JOURNAL OF AGRICULTURAL RESEARCH

VOL. XVIII WASHINGTON, D. C., NOV. 15, 1919 NO. 4

BACTERIAL BLIGHT OF SOYBEAN

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INTRODUCTION

For a number of years a bacterial blight of soybean has been under investigation at the University of Wisconsin. The early observations were made during several seasons prior to 1915 in the university experimental plots by Dr. L. R. Jones and Dr. A. G. Johnson, of the department of plant pathology. In September of that year more intensive study of the trouble was undertaken by the writer. At this time the disease was very severe in Madison fields. Scarcely a plant could be found free from the spotting, and the leaf area of approximately 20 per cent of the crop was destroyed to such an extent as to affect materially the growth of the plants. Subsequent observation has proved the blight to be very prevalent throughout the soybean fields of Wisconsin, and a disease showing symptoms of the same type has been reported from various other localities in the United States.

LITERATURE

Literature, up to this time, gives no detailed description of the disease or of its causal organism, although the malady has, no doubt, been observed in the field for many years. Smith [2] mentions a bacterial leaf-spot of soybean but does not record a thorough study of it. The only other references, with the exception of an abstract [3] published in 1917, seem to be a note by Heald [4] and a later report with figure by Clinton,[5] both of which give short descriptions of the symptoms. There seems no reason to doubt that these workers had under observation the disease described in this paper.

In addition it should be recorded that Manns [6] in 1915 described a bacterial organism pathogenic upon certain legumes, including soybeans.

[1] The writer wishes to make grateful acknowledgements to Dr. L. R. Jones and Dr. A. G. Johnson, of the University of Wisconsin, for supervision and helpful suggestions during the progress of this work.

[2] SMITH, Erwin F. BACTERIA IN RELATION TO PLANT DISEASES. V. 1, p. 92, 1905; V. 2, p. 69, 1911. Washington, D. C. (Carnegie Inst. Washington, Pub. 27.)

[3] JOHNSON, A. G., and COERPER, Florence M. BACTERIAL BLIGHT OF SOYBEAN. (Abstract.) *In* Phytopathology, v. 7, no. 1, p. 65. 1917.

[4] HEALD, Frederick De Forest. REPORT ON THE PLANT DISEASES PREVALENT IN NEBRASKA DURING THE SEASON OF 1905. *In* Nebr. Agr. Exp. Sta. 19th Ann. Rpt., 1905, p. 71. 1906.

[5] CLINTON, G. P. NOTES ON PLANT DISEASES OF CONNECTICUT. *In* Conn. Agr. Exp. Sta. Ann. Rpt., 1915, p. 441–446. 1916.

[6] MANNS, Thomas F. SOME NEW BACTERIAL DISEASES OF LEGUMES AND THE RELATIONSHIP OF THE ORGANISMS CAUSING THE SAME. Del. Agr. Exp. Sta. Bul. 108, 24 p., 21 pl. 1915.

He did not describe nor illustrate the disease on leaves, however; and although the spots on pods and stems, as shown in his plates, do not seem to be characteristically different in type from the lesions caused by the organism discussed in this paper, his report on cultural and morphological studies precludes the possibility of the two organisms being the same.

APPEARANCE OF THE DISEASE

On the leaves, where the disease is very conspicuous, the blight is characterized by small, angular lesions. When young these spots are translucent and water-soaked in appearance and yellow or light brown in color; when old they are dark reddish brown to almost black, very little of the translucency remaining. Single spots are usually from 1 to 2 mm. in diameter and may be quite generally scattered over the leaf surface, although they are often thickly grouped and confluent, resulting in irregular lesions several millimeters in diameter or even so large as to involve considerable portions of the leaf. Frequently, also, the spots seem to take the course of the principal leaf veins, and marginal infections are not unusual. Very often a yellowing of the tissue surrounding the lesion occurs. In advanced stages the invaded tissues may become dry and fall out, thereby giving the leaf a very ragged appearance (Pl. A).

Evidence all points strongly to the fact that the bacterial blight of soybean is not confined to leaves alone but that it affects also the stems, petioles, and pods. Black lesions on the stems and petioles, varying in size from very small spots to those of considerable length and breadth, are often found accompanying the trouble on the leaves, and water-soaked spots from which drops of exudate were oozing have also been found on young petioles (Pl. 12, A).

On the pods the disease appears first as small water-soaked spots, often showing exudate droplets (Pl. 12, B, C). The lesions may grow much larger, sometimes involving a considerable part of the pod. They usually turn to dark brown or black with age, the exudate drops frequently drying down as brownish nubs or scales. The seeds within diseased pods also become affected and may be found covered with a slimy bacterial growth (Pl. 13, A, B).

Isolations made from petioles and pods such as are here described have produced typical lesions when applied to soybean leaves, and re-isolations have yielded the typical organism.

Under the lens or even with the unaided eye, glistening exudate may often be observed in small quantities on the underside of the leaf spots. This ooze seldom appears as droplets in the field, except under very favorable moisture conditions, but infected tissue allowed to remain in a damp chamber for a number of hours is often covered with tiny drops of the grayish white exudate. These, however, on exposure to the air, dry very readily as inconspicuous brownish granules or scales, or seem to disappear entirely.

SEASONAL OCCURRENCE

The seasonal development of the bacterial blight has been under observation in a number of soybean fields in Wisconsin during the past four years, and a variety of conditions has been found to exist. Sometimes the disease will appear with the first leaves and progress steadily throughout the season. In other cases, the first leaves may show infection, then there may be a considerable amount of healthy growth, and later a further development of the disease on newer, younger tissue may occur. Sometimes, also, there has been no apparent development of the lesions until late July or early August, when the spotting might appear in abundance and continue until the plants were mature.

It seems reasonable to believe that weather conditions, especially those of moisture, have a great influence in determining the nature of the development and progress of the disease. However, since in the vicinity of Madison, Wis., all the above conditions have been observed in different fields during the same season, other factors than weather must have some importance in influencing the amount of disease present in any given locality. Just what these other factors may be is still an open question, since up to this time not enough experimental evidence has been accumulated to warrant making definite statements. The amount of seed infection suggests itself at once as having some relation, as do also the possibility of the persistence of the organism in the soil and the degree of exposure of the soybean plants to the blight organism. Moreover, field observations have shown that a real difference in varietal resistance exists. A further discussion of these relations will be given under the subject of control.

THE ORGANISM

ISOLATION

Microscopic observations of sections of invaded tissue show the interior of the lesions to be swarming with bacteria. The usual method of isolating the organism has been by the use of poured agar plates.

In the orignal isolation a portion of freshly invaded tissue was cut out, washed thoroughly through 10 changes of sterile water, and macerated upon a slide under as sterile conditions as possible. This material was introduced into sterile bouillon, further dilutions made, and plates poured, Thaxter's potato hard agar being the medium employed. In three days both yellow and white colonies had appeared on the plates. Characteristic colonies, both yellow and white, were picked and transfers made to potato agar slants. Water suspension inoculations with subcultures from several of these strains proved a white organism to be the pathogene. This conclusion was verified by subsequent re-isolation and re-inoculation. In no case did a yellow strain cause infection.

In later work the organism has been obtained with almost complete suppression of the yellow species, which are, no doubt, surface organisms, by dipping a piece of diseased tissue in 95 per cent alcohol for an instant, immersing in mercuric chlorid (1 to 1,000) for one minute, and then rinsing through five or six sterile water blanks before macerating and plating out.

When exudation has been abundant and the tissue clean, successful isolations have been also made by touching an exudate drop with a sterile platinum needle and transferring directly to an agar slant. This method has proved especially satisfactory when the exudate has been forced out in a damp chamber from leaves artificially infected in the greenhouse, although isolations have been made from field material in the same way.

FIG. 1.—*Bacterium glycinicolum*. From 2-hour growth on potato agar, stained by Duckwall's method to show flagella. × 2,000.

In the investigation of this disease a number of strains of the causal organism have been used in inoculation as well as in cultural studies. One of these, designated A, which has proved especially virulent in both greenhouse and field experiments, has been studied intensively and is presented as the type strain. This isolation was made from a leaf lesion in 1917. Some of the other isolations will be considered in a comparative way later in this paper.

MORPHOLOGY

The organism is a medium-sized rod with rounded ends and occurs usually singly or in pairs. When stained from 2-day-old potato agar cultures with Ziehl's carbol fuchsin or gentian violet, the cells average about 2.3μ long by 1.2μ wide. When stained with Duckwall's flagellum stain the average measurement is about 3μ by 1.5μ.

Both Zettnow's and Duckwall's flagellum stains have shown the organism to be motile by means of one to several polar flagella (fig. 1). No endospores or involution forms have been observed. Capsules were not demonstrated when stains were made from potato or beef-peptone agar cultures, but well-developed capsules were found present on blood agar. Both Welch's and Hiss's methods of staining were used. Light flocculent and sometimes membranous pellicles developed on the surface of certain liquid media such as bouillons of favorable reactions, sugar solutions, and on Fermi's and Uchinsky's solutions. The organism is gram negative.

CULTURAL CHARACTERS

Unless otherwise specified, all cultures were incubated at 25° C., a temperature very favorable for the growth of the organism. Reactions were determined by titration with phenolphthalein as indicator, the trial solution always having been boiled previous to test. All references to reaction of media are recorded in terms of Fuller's scale. Ridgway's color standards [1] were used in the determination of color.

AGAR POURED PLATES.—On potato agar, colonies appeared in about 48 hours and at the end of 5 days were from 2 to 5 mm. in diameter. They were creamy white tinged with brown, circular, shining, and convex with umbonate center. The margin in general was entire, although it may be slightly lobed. The surface may show indication of irregular wrinkling or beading in the central part, or there may be more or less concentric and radiating convolutions throughout the colony with a definite growth border.[2] The consistency was butyrous. Buried colonies were lenticular.

On +10 beef-peptone agar, colonies appeared in about 48 hours and at the end of 5 days were about 2 to 3 mm. in diameter. They were circular, smooth, shining, and convex. The margin was entire, with no noticeable surface irregularity. The colonies were creamy white in color tinged with brown, butyrous in consistency, and showed a definite browning of the medium around them. This brown color (chestnut) later became general throughout the entire plate. Buried colonies were lenticular.

AGAR STABS.—Stabs in potato agar when 2 days old showed a surface growth about 6 mm. in diameter, rather flat, shining, and creamy white tinged with brown. Later this growth may spread over three-fourths to almost the entire surface. Definite but moderate growth followed the stab. There was no change in the medium.

Stabs in +10 beef-peptone agar when 3 days old showed a surface growth about 5 mm. in diameter, rather flat, shining, creamy white tinged with brown, with slight browning of the medium to the depth of the stab. Later the top growth may become more spreading, involving a considerable part of the surface. Definite growth, a little more marked than on potato agar, followed the stab. The medium finally became quite uniformly browned.

AGAR SLANTS.—On potato agar slants, stroke cultures made a moderate, shining, flat, filiform to irregularly scalloped growth, creamy white in color tinged with brown. More or less wrinkling may occur on the surface. At low temperatures the growth was thicker and more

[1] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D.C., 1912.

[2] The irregularities are not always clearly evident to the naked eye. They show best under magnification and when lighting is semidirect. There is also considerable variation in the degree of surface marking of different strains of the blight organism (Pl. 15).

piled up, more shining and less wrinkled. The consistency was butyrous. Old cultures became dull, and the brownish tinge deepened.

On +10 beef-peptone agar, growth was slower and less abundant than on potato agar. It was also thinner, and of finer consistency. Surface irregularities were usually present as was the unevenly scalloped margin, though these characteristics were not always conspicuous. The color was creamy white tinged with brown. In about three days the agar began to take the chestnut-brown color observed in the plate and stab cultures. It was darker directly under the streak, and in a week the medium usually was uniformly browned. The agar discolored more slowly when cultures were grown at low temperatures or under adverse conditions.

GELATIN PLATES.—At room temperature (about 21° C.), the colonies were usually small on +10 peptone gelatin plates. There was no liquefaction of the medium, but it soon turned chestnut-brown in color.

GELATIN STABS.—At room temperature in +10 peptone gelatin, the surface growth was spreading after several days. There was no liquefaction, but the medium became a deep chestnut-brown about one-third of the distance down from the top. There was slight indication of growth following the stab.

POTATO CYLINDERS.—Growth on steamed potato cylinders in two days was spreading, rather flat, shining, slimy, and more or less viscid in consistency, smooth, without odor, and yellowish white in color. The growth turned the potato brownish gray.

MILK.—Inoculated milk coagulated slowly. In a month there was a fine curd developed, but no decided separation. The cultures took on a cream color and had a sort of half-transparent appearance. In two months a thin, somewhat clear layer developed on top, with the half-transparent-looking curd below.

LITMUS MILK.—Lavender-colored litmus milk cultures began to turn blue at the top three days after inoculation. In six days they were quite uniformly blue and remained without further change for about a month, when separation occurred. In seven weeks there had developed a thin, darker blue, rather clear layer on top, with fine, soft curd below and a small amount of cream-colored precipitate at the bottom.

METHYLENE BLUE IN MILK.—At the end of two days whitening began at the bottom and proceeded gradually upward. In about two weeks the cultures had become fairly white throughout with a blue layer at the top only. After this the blue color returned slowly, followed by a second whitening process which in about six weeks from the time of inoculation was complete, except for a thin layer at the top of the cultures. Curd began to form about this time, giving the cultures a half-transparent appearance. In two months there was a thin, somewhat clear, greenish blue layer on top with semitransparent, creamy, soft curd below. There was no marked separation.

NITRATE BOUILLON.—Nitrate bouillon cultures in fermentation tubes gave good clouding in the open arm but none in the closed arm. No gas was produced. In all cases there was a definite line of demarcation across the U of the tube. There was a fair positive test for ammonia and a strong positive test for nitrates. No nitrites were found. The medium in the open arm became so dark brown in color that it was impossible to test with any degree of accuracy for acidity.

FERMENTATION TUBES.—The tests were made in 2 per cent Difco peptone water and 2 per cent of each of the following carbon compounds: Dextrose, lactose, saccharose, maltose, glycerin, and mannit. Clouding was first observed at the end of 24 hours. In a week good growth had developed in all tubes in the open arm. With all of the sugars except dextrose, considerable cloudiness developed also in the closed arm, but no gas was found in any tube. Tests proved that there was growth in the closed arm of the dextrose culture tubes also; but here it was more or less slimy, and clear rather than cloudy. *Bacterium coli* was used as the control in this test, and gas was produced in all *Bact. coli* tubes.

When the cultures were 2 weeks old they were titrated, and they were tested again at the end of five weeks. Phenolphthalein was used as the indicator, and in every test there was preliminary boiling to drive off the carbon dioxid. At the end of two weeks the maltose, lactose, glycerin, and mannit cultures showed considerable increase in alkalinity. When 5 weeks old, however, the tests showed that acid was being produced again, and in some instances the cultures were more acid than the controls. The maltose and lactose cultures had turned so dark brown in the open arm that it was difficult to determine the exact endpoint in the titrations, but several trials made from each tube where the medium was not so dark-colored gave a satisfactory check on the results.

After two weeks' growth the dextrose and saccharose cultures showed, in general, increased acidity. In a number of instances, however, the tests made from the closed arms indicated an increase in alkalinity there. At the end of the 5-week period all the cultures showed an increase in acid in both open and closed arms.

The condition just described, which at first glance looks a bit confusing, may be explained by the following theory: The organism grows very rapidly in the dextrose and saccharose solutions where it at first causes an alkaline reaction, followed by the production of acid. The change does not take place so rapidly in the closed arm, and for this reason titration after two weeks still showed increased alkalinity. The open arm had gone beyond this point, and acid was being produced there in excess. Gradually, as we have seen, the acid condition became general throughout the tubes. No doubt the same action took place in the lactose, maltose, glycerin, and mannit cultures already described, except that there the changes occurred more slowly.

Nestler's reagent was used for the ammonia test. The maltose and lactose cultures gave a fair positive reaction; the other carbohydrates gave a very weak test for ammonia.

TITRATION OF SODIUM CHLORID.—Tubes of neutral, peptonized beef bouillon, containing, respectively, 0.5, 1, 1.5, 2, 3, and 4 per cent chemically pure sodium chlorid, were inoculated from 5-day-old potato agar cultures. In about a week there was fair clouding in the 0.5 per cent and the 1 per cent strengths. No growth occurred in any other tubes.

OPTIMUM REACTION AND TOLERATION LIMITS.—Peptonized beef bouillon was used for this test. Hydrochloric acid was the acid employed and sodium hydrate was the alkali. Bouillons were prepared, titrating +25, +22, +20, +18, +15, +10, +5, 0, −5, −10, −20, and −25, and were inoculated as uniformly as possible from 6-day-old potato agar cultures. At the end of 48 hours growth was visible in +18, +15, +10, +5, and 0; and in a week growth was very good in these cultures, with a light flocculent pellicle. Browning, working down from the top, began in these from the seventh to the ninth day, and the color continued to deepen to a reddish brown (chestnut) throughout. The +10 and +15 cultures were darker in color than the +5, and these in turn were darker than those at 0 and +18. The +10 culture showed heaviest growth. In a month +18, +15, +10, and +5 cultures showed no more clouding; but there was a precipitate at the bottom, especially heavy in the +10 tubes. This precipitate broke up readily on shaking. The 0 cultures at this time were still cloudy, but there was also a precipitate which broke up easily on shaking.

Growth developed slowly in the −5 reaction, browning also taking place more slowly than with the other cultures. A light, flocculent pellicle developed in some cultures. In a month these tubes showed various shades of brown, but they were no longer cloudy. There was a precipitate which broke up on shaking.

Slow growth took place in two tubes of +20, which also turned chestnut color in about a month, though they still remained slightly cloudy. There was a heavy precipitate which was easily broken up on agitating.

None of the other reactions showed growth.

Apparently, therefore, +10 Fuller's scale seems to represent the optimum reaction

FERMI'S SOLUTION.—Tubes of Fermi's solution inoculated from young potato agar cultures developed good clouding in 4 days. A flocculent pellicle began to form in about a week and was heavy in 14 days. Slight fluorescence appeared in about 2 weeks and later became fairly decided. The pellicle began to settle in about 5 weeks; and finally there was a heavy, flaky, and somewhat stringy precipitate at the bottom of the cultures.

USCHINSKY'S SOLUTION.—Inoculation was made from young potato agar cultures. Clouding was apparent in 4 days and was heavy in 6

days, with a light, fluocculent pellicle forming, which later became heavy. Fluorescence appeared in about 10 days and persisted throughout the entire test, 7 weeks. At the end of this time the pellicle had disappeared; but there was a heavy, flaky precipitate at the bottom of the cultures.

COHN'S SOLUTION.—The organism grew slowly in Cohn's solution. No fluorescence appeared.

STARCH AGAR.—There was no evidence of diastatic action on potato starch suspended in beef-peptone agar, when tested with potassium iodid-iodin.

INDOL.—Erlich's test for indol was used with +10 beef-peptone bouillon as the medium. There was a weak, positive reaction from the third to the seventh day. After this time the cultures had grown too dark to give the color test.

BLOOD SERUM.—Stroke cultures on solidified blood serum gave a moderate, rather flat growth, smooth, shining, and creamy white tinged with brown. The medium was not liquefied.

AEROBISM.—The organism behaved as a weak facultative anaerobe. Clouding occurred in the closed arm of the fermentation tubes with all the sugars tested except dextrose. Definite though not vigorous growth occurred below the surface in stab cultures. Stroke cultures of agar also gave indication of slight growth for some distance below the surface.

LITMUS AGAR WITH SUGARS.—Litmus-lactose and litmus-maltose stroke cultures developed good growth. In six days the medium began to brown under the streak and finally turned a rich reddish brown throughout. This is the same sort of change that takes place in all beef-peptone media. There was no apparent reddening or bluing due to the predominating production of acid or alkali, but, judging from the test with carbohydrates in fermentation tubes, it is probable that such changes took place and were masked by the browning of the medium.

On litmus-dextrose agar there was abundant growth and a distinct acid reaction. In seven days the cultures had become decidedly scarlet. In about six weeks the red color disappeared again, leaving the cultures about the same color as the controls.

TEMPERATURE RELATIONS

Cultures on potato agar and in +10 beef-peptone bouillon proved the optimum temperature to be between 24° and 26° C. No growth occurred at 35° either in bouillon or on agar. Rather slow but fairly heavy growth occurred both on agar and in bouillon at 2°. The extreme minimum was not determined.

Several trials have shown the thermal death point of 2-hour cultures made from 2-day-old cultures to be 48° to 49° C. for freshly isolated, vigorous strains. Consistent results have been secured in trials of three different years. Isolations which have been cultured for some time,

however, show that there is a gradual lowering of the death point with age. In a recent test (1919) it was found that cultures from a 1915 strain were practically all killed at 46° to 47°, whereas trials with this same strain in 1916 had shown the normal death point to be 48° to 49°. In the 1919 trials all tubes of a 1917 isolation developed fair growth after being subjected to 47°, but there was no growth after 48°. Cultures of a 1918 strain showed good growth in a number of the tubes after 48°, although clouding was not uniform throughout. In no test, however, has there been any clouding after 49° or above.

DESICCATION

The organism seems not to be extremely resistant to drying as it occurs in the host tissue. Isolations attempted from herbarium material 1 and 2 years old failed to yield the organism. The vitality varies on different culture media. On potato agar, where growth is very abundant, the organism usually dies within two months, whereas on beef-peptone agar and in bouillon it lives considerably longer. Transfers made from 6-months-old cultures of these last-named media developed good growth. Also cultures kept at a low temperature in a moist atmosphere, such as that afforded by an ice box, seemed to retain their vitality for a much longer time than those allowed to remain at a higher temperature in a dry incubator.

When dried on sterile cover glasses the organism was found to live only a comparatively short time. In these tests a young, vigorously growing bouillon culture was diluted with an equal amount of sterile water and a 2-mm. loop of this dilution transferred to sterile cover glasses which were then dried in a sterile chamber. When these cover glasses were dropped at intervals into tubes of sterile bouillon, it was found that the organism was not alive after 65 hours of drying.

TECHNICAL DESCRIPTION

Bacterium glycineum n. sp.[1]

Cylindrical rods, rounded at ends, solitary or in pairs; individual rod 2.3 to 3μ by 1.2 to 1.5μ; motile by 1 to several polar flagella; aerobic to weakly anaerobic; no spores; capsulated when grown on blood agar.

Superficial colonies on potato agar plates round, shining, convex with umbonate center and surface irregularities; creamy white tinged with brown; margin slightly lobed.

No liquefaction of gelatin; does not digest casein; nitrates not reduced; cultures in various carbohydrate media produce acid with no gas. Gram negative.

Group number, 222.2223032.

Pathogenic on *Glycine hispida* Maxim., forming angular lesions which are seriously destructive to leaves. Affects also pods and other aerial parts.

Type locality: Madison, Wis., on *Glycine hispida* Maxim.

Distribution: Widespread.

[1] According to Migula's classification, the combination would be *Pseudomonas glycineum*, n. sp.

VARIATIONS AMONG STRAINS

As has already been suggested, a number of other strains of the blight organism have been isolated and studied in comparison with strain A, and during the progress of this work certain interesting variations have been found to exist.

The original isolation, strain E, was obtained from a leaf lesion in the fall of 1915, was studied in the greenhouse and culturally in the laboratory during 1916, and has been used in comparison with the type strain, A, in all inoculation and culture work since that time. Its original pathogenicity was proved; but its virulence, which was never so great as that of A, seems to be practically lost after three years.

In culture, strains A and E behave very much alike, but with one marked difference. It has been shown that strain A causes a consistent browning of certain media, such as beef-peptone broth, beef-peptone agar, and peptone solution plus certain sugars; whereas strain E does not cause this color change. Also, strain E, under favorable condition for growth, develops, on potato agar, colonies and streak cultures showing a decidedly wrinkled and convoluted surface. On the other hand, while colonies and slope cultures of strain A, grown on potato agar, may develop a more or less irregular surface also, ordinarily they appear much smoother than those of E and do not make such an abundant growth. There is in addition a slight color difference, strain A showing on potato agar a browning tinge which is absent in E until the cultures are rather old.

A number of isolations made during the seasons of 1917 and 1918 from leaf, petiole, and seed lesions correspond with strain A in color and in the ability to brown peptone media. In general, colonies and slope cultures of these isolations, on potato agar, compare more favorably with strain E in surface irregularity, although there are smooth types among them. These later strains have not been studied in detail, but they have all proved to be pathogenic on soybean leaves and are apparently the same as the type leaf strain. The writer also has in reserve certain isolations made from stem and leaf which correspond with the original strain E in grosser cultural characters and in the apparent inability to cause browning of the peptone media. It is hoped that the pathogenicity of these strains may be determined during the coming season. One strain of this type, recently isolated from a leaf which developed natural infection in the greenhouse, has produced typical lesions on soybean seedlings.

On beef-peptone agar no structural differences between colonies and streaks of different strains appear. Here we find simply the difference in ability to brown the medium. Plates 14 and 15 show the lack of uniformity in the surface characteristics of colonies and slope cultures of pure, authentic strains of the soybean blight organism. It will be

noted that considerable variation may occur among colonies on the same plate.

Just what the significance of these variations may be is rather difficult to state without more thorough study of this part of the cultural work. We have found, however, that both types of the organism are pathogenic on soybean leaves and are able to produce typical lesions. Furthermore, there have been no marked differences in morphology or in cultural behavior. It seems, therefore, the justifiable disposition of the matter, at least for the present, to consider that we are dealing with forms of a slightly variable species. Since these forms agree in the characters ordinarily included in descriptions of bacterial species as well as in pathogenicity and host reactions, it does not seem wise nor helpful to segregate them formally by technical description or varietal name. Should subsequent investigations indicate more adequate bases or need for this segregation it may then be made.

INOCULATION EXPERIMENTS

During the years 1916 to 1918, the bacterial leaf spot has been reproduced many times and in typical form under greenhouse and field conditions by artificial inoculation (Pl. 16, 17). From lesions produced in this way, the original type organism has been repeatedly recovered. In the early work the tissues were wounded at the time of inoculation, but it was soon found that simply spraying water suspensions of the blight organism upon uninjured leaves was sufficient to produce infection, not only when the tissue was very young and tender but also when the leaves had more nearly approached maturity. It has been found advisable, however, in most cases, since soybean leaves are very hairy and therefore prevent liquid from spreading readily upon them, to rub the inoculated leaves gently between thumb and finger in order to insure contact between the inoculum and the leaf surface.

In making greenhouse inoculations the following method was usually employed: Soybeans were sown in 8-inch pots in sterile soil, and when a few leaves had developed they were inoculated with an atomizer spray of a water suspension of the organism. The plants were then allowed to remain in a damp chamber for from 48 to 72 hours, after which they were removed to a greenhouse bench.

In making inoculations in the field, practically the same method was employed. Plants perfectly free from the disease were selected, the leaves inoculated with atomizer spray and then covered with glasine paper bags which were removed after two or three days. In field work the younger leaves of almost mature plants were most often used in the inoculation tests.

Control plants were always sprayed with sterile water and otherwise treated in the same manner as inoculated plants. Both inside and out

of doors typical lesions usually began to develop in about 10 days after the inoculum was applied.

Only one attempt was made to inoculate pods. This experiment was performed in the field in September, 1917, when young pods were simply sprayed with a water suspension of strain A. Typical lesions developed in almost every pod, whereas the controls remained absolutely free from spots (Pl. 13, B).

RELATIONS TO HOST TISSUE

Razor sections of lesions in fresh leaves show the bacterial invasion to be in the parenchymatous tissue. Critical histological studies have not been made, but leaf lesions fixed in Gilson's fixative, embedded in paraffin, sectioned, and stained in carbol fuchsin have shown intercellular invasion. Infection evidently takes place through the stomata.

OVERWINTERING, DISSEMINATION, AND CONTROL

Practically no experimental work has been done up to this time concerning the bacterial blight organism in relation to overwintering and dissemination. It has been noted, however, that the disease appears year after year in the same field and also that it may appear almost as soon as the first leaves have developed, although it must be remembered that this latter condition is not at all universal and that the lesions sometimes make their first appearance when the plants are nearing maturity. These facts seem to indicate that the organism may be carried with the seed and also that it may live over in the soil. Pods and seeds often become badly affected with the disease as we have already seen and, although we have learned that the organism does not withstand long periods of desiccation, at least in the leaves, there is a possibility that it may live a much longer time upon the seed, or, judging from the nature of the invasion, within it.

Until further work on overwintering and dissemination has been done it will be impossible to recommend any specific control measures aiming to check the development and spread of the disease in the field. Destroying all diseased plants, rotation, and the selection of sound seed for planting have been suggested as possible means of control; but it will be seen at once that the first recommendation is impracticable from the standpoint of soybean culture; and, considering how generally the disease occurs, we can not entertain very great hopes of the other two in practice. Considerable observational data have been accumulated, however, during the last few years concerning varietal resistance and susceptibility to the soybean blight, and there seems to be no question that decided differences in varietal resistance exist. During the last few seasons the writer has observed repeatedly, in variety test plots, perfectly healthy plants growing immediately adjacent to others

almost completely destroyed by the bacterial blight. In fact, very often the leaves of the two plants were in actual contact and yet this difference in susceptibility to the disease persisted throughout the season. Inoculation experiments in the greenhouse have strengthened the belief that certain varieties of soybeans are immune to the attacks of the blight organism (Pl. 18), and organized experimental work is now under way at the University of Wisconsin on this phase of the problem. It seems very probable from evidence at hand that the bacterial blight might at present be greatly mitigated if not satisfactorily controlled by the intelligent selection of disease-resistant varieties from among those already in use. If it appears that the ideal combination of disease resistance with other desirable characters does not occur in the standard varieties now preferred, it should be possible to combine these by properly directed breeding efforts.

SUMMARY

(1) The blight of soybean described in this paper has been observed in the University of Wisconsin plots for several years. A disease, undoubtedly the same, has been reported from various parts of the United States.

(2) It is characterized on the leaves, where it is most conspicuous, by small, angular spots, either isolated or confluent. The lesions are light-colored and translucent in early stages and very dark-colored in late stages. In late stages, also, the diseased tissue may become dry and drop out, giving the leaves a ragged appearance. Bacterial exudate occurs on the leaf spots as droplets but is not very evident except under favorable moisture conditions. It is pale in color and dries as inconspicuous granules or scales.

(3) Petiole, stem, and pod lesions accompany the disease on the leaves. Exudation has been observed on petioles and pods.

(4) The blight is caused by *Bacterium glycineum*, n. sp., which is able to make entrance into the tissues without wounds. The organism is a medium-sized rod, motile by means of from one to several polar flagella. In culture its color is creamy white tinged with brown.

(5) Isolation of the organism has been accomplished repeatedly by macerating a thoroughly washed lesion upon a slide under sterile conditions and using this material for dilutions from which agar plates were poured. Cultures made by transferring from characteristic colonies or from fresh exudate droplets to agar slants have furnished the material used in the inoculation work.

(6) Simply spraying water suspensions of the organism upon soybean plants is sufficient to produce infection. It is advisable, however, to rub the tissue gently between thumb and finger to insure contact between inoculum and plant surface.

(7) Best growth of the organism occurs between 24° and 26° C. The maximum is about 35°. The absolute minimum has not been determined. Slow growth develops at 2°.

(8) The organism is sensitive to desiccation, and there seems to be gradual loss in pathogenicity when it is grown in artificial culture.

(9) Certain variations in cultural behavior exist between different strains of the soybean blight organism, including the ability of some strains—for example, the type—and inability of others to brown the peptone media. Studies to date have led us to consider, at least for the present, that we have been dealing with forms of a slightly variable species.

(10) Invasion occurs in the parenchyma.

(11) Control measures are not yet fully worked out. At present there seems to be greatest promise in the development of disease-resistant varieties.

PLATE A

Bacterial spot on leaves of soybean.[1]

[1] This plate was prepared under the direction of Dr. M. W. Gardner, Bureau of Plant Industry, United States Department of Agriculture.

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PLATE 12

Bacterium glycineum:

A.—Soybean petiole. Natural infection, showing exudate droplets. × 6.

B.—Blighted soybean pod. Natural infection, showing young water-soaked lesions. × 6.

C.—Blighted soybean pod. Natural infection, showing exudate droplets. × 6.


B
C
A


A
B

PLATE 13

Bacterium glycineum:

A.—Natural infection on soybean pods. Note also seed infection in pod at right. × 1.

B.—Artificial infection on soybean pods inoculated with type strain. × 2.

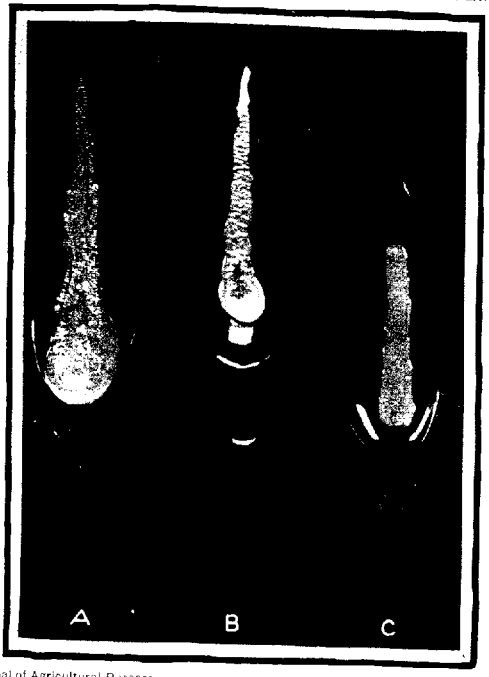
PLATE [illegible]

Bacterium glycineum:

A.—Six-day-old potato agar slant culture, strain E.

B and C.—Six-day-old potato agar slant cultures, type strain. Note difference in degree of surface marking.

PLATE 14



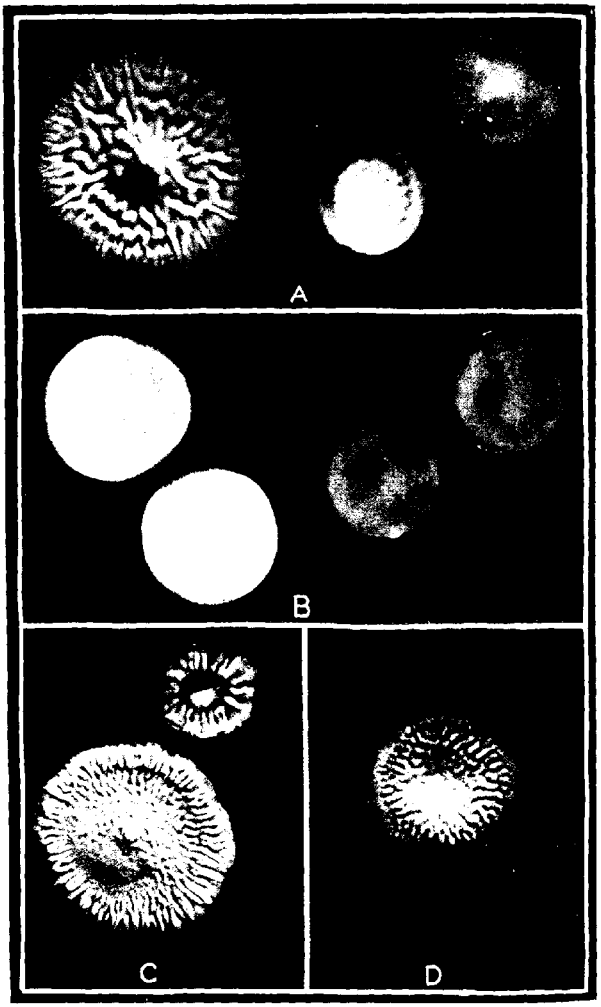

A
B
C
D

PLATE 15

Bacterium glycineum:

A.—Colonies of petiole strain on potato agar plate.

B.—Colonies of type strain on potato agar plate. Surface irregularities present but not conspicuous.

C.—Colonies of strain E on potato agar plate.

D.—Colony of seed strain on potato agar plate. All plates were dilution cultures from bouillon. Note the differences in surface marking even among colonies on the same plate.

PLATE 16

Bacterium glycineum.

Soybean seedlings showing infection after inoculation in the greenhouse with type strain. Control plant at right.

PLATE 17

Bacterium glycineum:

Blighted soybean leaves artificially inoculated in the field with type strain. The two leaves at the top of the page were punctured previous to use of atomizer spray; the center leaf was simply sprayed; the two lower leaves were sprayed and then rubbed lightly.

PLATE v8

Bacterium glycineum:

Soybean seedlings artificially inoculated in the greenhouse with type strain. Two plants at left, susceptible variety. Plant at right, resistant.

DOMOLD SPORES CONTAIN ENZYMS?

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Molds have long been known to produce intracellular and extracellular enzyms, but so far as we have been able to ascertain no study has been made of the enzyms contained in the spores of these organisms. This is indeed noteworthy, since the valuable contributions of Duclaux, Fernbach, and others mentioned by Effront[2] and Dox[3] have apparently been concerned solely with the enzymic activities of molds in the mycelial stage. It has been shown by us in another connection[4] that the deterioration of sugar occurred where mold mycelia developed and in certain instances where spores alone were present. This phenomenon gave rise to the query, Do mold spores contain enzyms? This is the concern of the present investigation. It is our purpose to limit the scope of this article to the invertase activity of the spores of *Aspergillus niger* (*Aspergillus sydowi* [Bain. and S.]), *Aspergillus flavus*, and *Penicillium expansum*, because of the economic significance of this enzym and the wide distribution of these molds.

METHOD OF PROCEDURE

A large number of Petri dishes containing Kopeloff's agar[4] were seeded with a single pure, bacteria-free culture of the desired mold and incubated at 35° C. for six days. A small amount of sterile distilled water was introduced into each plate, shaken slightly, and then poured on sterilized filter paper (Whatman No. 4). The mycelium was then washed through the filter paper with sterile distilled water, and the spores were separated by a combination of filtration and flotation. This process was continued long enough to accumulate the desired quantity of spores. The spores were then rinsed off the filter paper into a sterile Erlenmeyer flask of 300-cc. capacity. To 250 cc. of this spore suspension and an equal volume of sterile distilled water, 1 cc. of c. p. chloroform was added.

[1] The authors are indebted to Director W. R. Dodson, Assistant Director W. G. Taggart, Dr. F. W. Zerban, and Mr. E. C. Freeland for their kind interest and assistance, and to Mr. W. L. Owen for reading the manuscript.

[2] EFFRONT, Jean. ENZYMES AND THEIR APPLICATIONS . . . English translation by Samuel C. Prescott. 322 p. New York, 1902.

[3] DOX, A. W. THE INTRACELLULAR ENZYMES OF PENICILLIUM AND ASPERGILLUS . . . U. S. Dept. Agr. Bur. Anim. Indus. Bul. 120, 70 p. 1910.

[4] KOPELOFF, Nicholas, and KOPELOFF, Lillian. THE DETERIORATION OF CANE SUGAR BY FUNGI. La. Agr. Exp. Sta. Bul. 166.

An examination of the number of spores present per cubic centimeter was made by means of the blood-counting cell, as described by us in another connection.[1] Unfortunately it was impossible to disintegrate the clumps, and the reported counts are consequently approximate. In this instance there were about 2,000,000 spores per cubic centimeter of inoculum. The flasks were then heated to 63° C. for 30 minutes for the purpose of killing the spores[2] and making the spore wall more permeable. The contents of each flask were then transferred to Erlenmeyer flasks containing sterile washed sand and shaken vigorously for 5 minutes in order to cause further rupture in the spore walls. The inoculum was then ready to be added to the 200-cc. portions of 10 per cent (by weight) granulated sugar solution in cotton-plugged Erlenmeyer flasks, which had been previously sterilized in the autoclave at 15 pounds pressure for 15 minutes. All the flasks had the same polarization. Ten cc. of sterile distilled water were added to the control flasks, and 10 cc. and 20 cc. of inoculum were added to the others. After this inoculation both the inoculum and sterile distilled water were heated to 100° for 20 minutes to kill any enzym which might be present. Then this sterilized inoculum was added to the sugar solution as explained above. All flasks were then incubated at 35° to 45° for 3 hours. At the end of this time 50 cc. of solution were removed with a sterile pipette, filtered, and polarized. Reducing sugars were determined in the same sample by the modified Violette method (volumetric). The results are recorded in Table I. The actual polarization values and the average percentage of decrease in sucrose are given in separate columns.[3]

It will be seen from the results in Table I that the flasks inoculated with sterile distilled water and those inoculated with sterilized inoculum, heated to 100° C., had the same polarization (35.7), within experimental error, and the same amount of reducing sugars (0.04 per cent), while the flasks having 10 cc. of spores heated to 63° polarized 34.8, which represents a loss in polarization of 0.9 as compared with the control, or a decrease of 0.23 per cent sucrose. The reducing sugars increased 0.02 per cent. The inoculum of spores was polarized alone and exhibited no optical activity. However, where 20 cc. of spores heated to 63° were used, the polarization dropped to 34.1, which was a decrease of 1.5 as compared with the control, or an actual decrease of 0.38 per cent sucrose. The reducing sugars were slightly increased. Where 20 cc. of inoculum were used it is necessary to correct for the added dilution, if we wish to compare these results with those obtained with 10 cc. of inoculum.

[1] Kopeloff, Nicholas, and Kopeloff, Lillian. Op. cit.

[2] Thom, Charles, and Ayers, S. Henry. Effect of pasteurization on mold spores. *In* Jour. Agr. Research, v. 6, no. 4, p. 153-166, 3 fig. 1916.

[3] The authors wish to thank Mr. E. C. Freeland, Assistant Chemist, for his kind assistance with these analyses.

Furthermore, it must be remembered that throughout these results each polarization value represents the optical activity of all three sugars present—sucrose, dextrose, and levulose—and consequently can not be accepted unreservedly as an adequate criterion of inversion; rather we must consider the reducing sugars for such a purpose. From these results it is evident that after three hours the enzymic activity of the spores of *Aspergillus niger* was already manifest. A microscopic examination of the contents of each flask was made for the purpose of detecting any germination or bacterial contamination, if present. No germination or contamination occurred. Thus the enzymic activity, as evidenced by the reduction in polarization and increase in reducing sugars, was produced by the spores of the mold present. That this inversion was truly enzymic is proved by the fact that when the spores were heated to 100°, which temperature kills all enzym activity, the inoculation induced no change in the composition of the original sugar solution.

TABLE I.—*Analyses of 10 per cent sugar solutions inoculated with spores of Aspergillus niger*

[Three hours' incubation]

Flask No.	Treatment.	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
				Per ct.	*Per ct.*	*Per ct.*
3	10 cc. sterile water heated to 63° C.	35.6	.....	.....	0.03	.....
4	.....do.....	35.6	.....	.....	.04	.....
	Average.....	35.6	.....	.....	.04	.....
12	10 cc. sterile water heated to 100° C.	35.8	.....	.....	.04	.....
14	.....do.....	35.8	.....	.....	.04	.....
	Average.....	35.8	.....	.....	.04	.....
5	10 cc. spores heated to 100° C.	35.8	.....	.....	.04	.....
6	.....do.....	35.6	.....	.....	.04	.....
	Average.....	35.7	.....	.....	.04	.....
15	10 cc. spores heated to 63° C.	34.9	.....	.....	.05	.....
16	.....do.....	34.8	.....	.....	.06	.....
17	.....do.....	34.8	.....	.....	.07	.....
	Average.....	34.8	0.9	0.23	.06	0.02
18	20 cc. spores heated to 63° C.	32.0	.....	.....	.07	.....
19	.....do.....	32.3	.....	.....	.06	.....
20	.....do.....	32.1	.....	.....	.07	.....
	Average.....	32.1	3.6	.90	.07	.03
	Corrected to 10 cc.	34.1	1.5	.38	.07	.03

TABLE II.—*Analyses of 10 per cent sugar solutions inoculated with spores of Aspergillus niger*

[Twenty-four hours' incubation]

Flask No.	Treatment.	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
				Per ct.	*Per ct.*	*Per ct.*
3	10 cc. sterile water heated to 63° C.	35.6	.....	.....	0.05	.....
4	.....do.....	35.6	.....	.....	.05	.....
	Average.....	35.6	.....	.....	.05	.....
12	10 cc. sterile water heated to 100° C.	35.8	.....	.....	.....	.....
14	.....do.....	36.0	.....	.....	.04	.....
	Average.....	35.9	.....	.....	.04	.....
5	10 cc. spores heated to 100° C.	35.8	.....	.....	.04	.....
6	.....do.....	35.8	.....	.....	.04	.....
	Average.....	35.8	.....	.....	.04	.....
15	10 cc. spores heated to 63° C.	31.1	.....	.....	.20	.....
16	.....do.....	31.0	.....	.....	.20	.....
17	.....do.....	31.0	.....	.....	.20	.....
	Average.....	31.0	4.6	1.16	.20	0.15
18	20 cc. spores heated to 63° C.	26.1	.....	.....	.44	.....
19	.....do.....	25.9	.....	.....	.50	.....
20	.....do.....	26.0	.....	.....	.50	.....
	Average.....	26.0	9.6	2.41	.48	.43
	Corrected to 10 cc.	27.2	8.4	2.11	.50	.45

The flasks each received three drops of chloroform and were returned to the incubator at 45° C., where they remained until analyzed at the end of the 24-hour incubation period. They were frequently shaken to assist in the rupturing of the spore walls. From the results recorded in Table II it will be noted that, as in Table I, the flasks inoculated with sterile water heated to 63° and 100° as well as the sterilized inoculum—spores heated to 100°—polarized about 35.8 and contained about 0.04 per cent reducing sugars. On the other hand, there was a marked decrease in polarization in the flasks inoculated with 10 cc. of spores heated to 63°. This is shown by the value 31, which represents a total loss of 4.6 or a decrease of 1.16 per cent sucrose. The reducing sugars increased appreciably to 0.15 per cent from 0.05 per cent. Still more striking is the fact that double this quantity of spores (20 cc.) was responsible for a proportional decrease in polarization—amounting to 8.4, or a decrease of 2.11 as compared with the control—and a slightly greater increase in reducing sugars. Again a microscopic examination disclosed no signs of

germination or contamination. Thus the results after 24 hours confirmed those obtained after 3 hours' incubation, and we must conclude that the spores of *Aspergillus niger* had released sufficient of the enzym invertase to carry forward the inversion of sucrose already noted.

Finally, after the addition of three drops of chloroform and another incubation, the flasks were analyzed at the end of four days. The results are to be found in Table III.

TABLE III.—*Analyses of 10 per cent sugar solutions inoculated with spores of Aspergillus niger*

[Four days' incubation]

Flask No.	Treatment.	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
				Per ct.	*Per ct.*	*Per ct.*
3	10 cc. sterile water heated to 63° C.	37.3	.....	.....	0.05	.....
4	.....do.....	37.2	.....	.....	.05	.....
	Average.....	37.3	.....	.....	.05	.....
12	10 cc. sterile water heated to 100° C.	36.8	.....	.....	.04	.....
14	.....do.....	36.8	.....	.....	.04	.....
	Average.....	36.8	0.5	0.12	.04	.....
5	10 cc. spores heated to 100° C.	37.0	.....	.....	.04	.....
6	.....do.....	36.8	.....	.....	.04	.....
	Average.....	36.9	.4	.09	.04	.....
15	10 cc. spores heated to 63° C.	23.9	.....	.....	.98	.....
16	.....do.....	24.7	.....	.....	.95	.....
17	.....do.....	23.7	.....	.....	.87	.....
	Average.....	24.1	13.2	3.31	.93	0.88
18	20 cc. spores heated to 63° C.	18.2	.....	.....	1.84	.....
19	.....do.....	17.9	.....	.....	1.90	.....
20	.....do.....	18.2	.....	.....	2.27	.....
	Average.....	18.1	19.2	4.80	2.00	1.05
	Corrected to 10 cc.	18.9	18.4	4.55	2.09	2.04

Compared with the previous analyses the control flasks—sterile water heated to 63° and 100° C. and spores heated to 100°—show a slightly higher and somewhat less consistent polarization. This is undoubtedly due to evaporation, the flasks having been plugged with cotton. That the discrepancies are negligible is evidenced by the uniformity in amount of reducing sugars present, which represent the true criterion of inversion. Where 10 cc. of spores heated to 63° were employed, the polarization was reduced to 24.1 as compared with 37.3 in the control, a loss of 13.2 or a decrease of 3.31 per cent sucrose. The reducing sugars indicate

a correspondingly high increase of 0.88 per cent. Where 20 cc. of inoculum were used it will be observed that the polarization was reduced 18.4 as compared with the control, or a decrease of 4.55 per cent sucrose, and the reducing sugars were increased to 2.04 per cent. Again no germination or contamination could be detected in a microscopic examination.

Since the particular problem which gave rise to the present investigation—namely, the deterioration of sugar—is concerned primarily with solutions of high concentration, it was planned to corroborate the above evidence by repeating the experiment and using a sugar solution of 20 per cent by weight, or double the concentration of that previously employed. The methods and technic were identical with those described in the experiment just reported, except that in this case there were fewer spores present in the inoculum.

TABLE IV.—*Analyses of 20 per cent sugar solutions inoculated with spores of Aspergillus niger*

[Twenty-four hours' incubation]

Treatment.	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 63° C.	61.0	.....	.....	0.05	.....
10 cc. sterile water heated to 100° C.	61.2	.....	.....	.05	.....
10 cc. spores heated to 100° C.	62.2	.....	.....	.05	.....
10 cc. spores heated to 63° C.	58.0	3.0	0.72	.38	0.33
20 cc. spores heated to 63° C.	52.9	8.1	1.94	1.21	1.16

In Table IV are recorded the results of the analyses after 24 hours' incubation at 45° C. Only the averages of closely agreeing triplicate determinations are here presented, and corrections for amount of inoculum are calculated. It will be observed that the polarization and reducing sugars of the flasks receiving 10 cc. of sterile distilled water heated to 63° and those heated to 100° were the same Ten cc. of spores heated to 100° gave a slightly higher polarization, but these variations are probably the results of the sterilization process, which did not affect all flasks in an identical manner. However, there is no question concerning the reduction in polarization as effected by 10 cc. of spores heated to 63°, which amounts to 3, or a decrease of 0.72 per cent sucrose. The reducing sugars show a somewhat greater increase—0.33 per cent. Where 20 cc. of spores were employed, the polarization was 52.9 as compared with 61 in the control flasks, a reduction of 8.1 or a decrease of 1.94 per cent sucrose. Correspondingly there was an increase in reducing sugars of 1.16 per cent. No germination or contamination could be detected by a microscopic examination. Thus the results obtained with a 20 per cent sugar solution confirmed those previously obtained

with the 10 per cent solution and form an adequate basis for establishing the fact that the spores of *Aspergillus niger* contain invertase.

TABLE V.—*Summary of enzymic activity of spores of Aspergillus niger*

Quantity of spores.	Concentration of sugar solution.	Incubation period.	Decrease in polarization.	Decrease in sucrose.	Decrease in sucrose.[a]	Increase in reducing sugars.	Increase in reducing sugars.[a]
Cc.	*Per cent.*	*Hours.*		*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
10.....	10	3	0.9	0.23	2.5	0.02	50
10.....	10	24	4.6	1.16	13.0	.15	300
10.....	20	24	3.0	.72	4.9	.33	660
10.....	10	96	13.2	3.31	35.4	.88	1,760
20[b].....	10	3	1.5	.38	4.2	.03	75
20[b].....	10	24	8.4	2.11	21.9	.45	900
20[b].....	20	24	8.1	1.94	13.3	1.16	2,320
20[b].....	10	96	18.4	4.55	50.0	2.04	4,080

[a] Original considered as 100 per cent. [b] Corrected to 10 cc.

In Table V the data previously presented are summarized in such a way as to make evident the correlations which are of interest. For example, it will be readily observed that with a given quantity of inoculum in a sugar solution of definite concentration there is a progressive decrease in polarization accompanied by an increase in reducing sugars with an increase in the incubation period. Again, it may be noted that increasing the inoculum causes practically a proportional decrease in polarization and a corresponding increase in reducing sugars. This lends further support to the evidence brought forward that spores contain enzyms, for it is precisely under such circumstances that a relationship is established between the number of spores and the amount of enzymic activity. While the data presented do not establish such a correlation with mathematical accuracy, one may assume that the number of spores whose walls become ruptured would be somewhat variable in quantity, and consequently a progressive increase in enzymic activity may be seen to accompany an increase in the number of spores.

It is especially interesting to note that 20 cc. of spores reduced the polarization of a 10 per cent sugar solution in 4 days in such a way as to indicate the loss of 50 per cent of the original sucrose present. This inoculum accounted for the loss of 22 per cent of the original sucrose content in 24 hours. The increase in reducing sugars is even more striking, as seen in the last column of Table V, where, in the cases mentioned above, the amounts were increased 4,080 and 900 per cent, respectively, compared with the original percentage taken as 100.

When the inoculation of a 10 per cent sugar solution with 10 cc. of spores is compared with a similar inoculation of a 20 per cent sugar solution, it will be seen that in 24 hours the amount of reducing sugars in the latter case was about double that in the less concentrated solution.

A similar phenomenon is to be observed when 20 cc. of spores are employed. This is in line with the theoretical considerations of the activity of the enzym invertase and indicates that the amount of inversion depends upon the quantity of sucrose present. The polarization values do not permit of such clearly defined generalization because of the more complex nature of the factors involved.

The procedure described above was repeated, using the spores of *Penicillium expansum* as an inoculum; and the results are recorded in Table VI. The calculations for diminished and increased dilution depending upon the amount of inoculum added have been incorporated.

TABLE VI.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Penicillium expansum*

Treatment.	After three hours' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
10 PER CENT SUGAR SOLUTION					
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 73° C.	38.5	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	38.5	.....	.....	.04	.....
10 cc. spores heated to 100° C.	38.5	.....	.....	.04	.....
5 cc. spores heated to 63° C.	38.5	.....	.....	.04	.....
10 cc. spores heated to 63° C.	38.5	.....	.....	.04	.....
20 cc. spores heated to 63° C.	38.4	0.1	0.02	.06	0.02
20 PER CENT SUGAR SOLUTION					
10 cc. sterile water heated to 63° C.	73.2	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	73.2	.....	.....	.04	.....
10 cc. spores heated to 100° C.	73.2	.....	.....	.04	.....
5 cc. spores heated to 63° C.	73.2	.....	.....	.06	0.02
10 cc. spores heated to 63° C.	73.0	0.2	0.05	.15	.11
20 cc. spores heated to 63° C.	72.6	.6	.15	.23	.19

It will be readily observed that with 5 and 10 cc. of inoculum no inversion took place in a 10 per cent sugar solution, and only a very slight increase in reducing sugars is to be found where 20 cc. were used. The inoculum contained 600,000 spores per cubic centimeter and exhibited no optical activity. However, where a 20 per cent solution was employed the reducing sugars increased with an increase in inoculum, while there was a substantial decrease in polarization and percentage of sucrose with the largest inoculum.

The increase in inversion which occurred with an increase in incubation period is not large and is in agreement with the facts which were noted after three hours' incubation. Thus it may be inferred that the spores of *Penicillium expansum* have a relatively slight invertase content.

The analyses at the end of 24 hours and 3 days, respectively, are presented in Tables VII and VIII.

TABLE VII.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Penicillium expansum*

10 PER CENT SUGAR SOLUTION

Treatment.	After 24 hours' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 63° C.	38. 5	.....	.....	0. 04	.....
10 cc. sterile water heated to 100° C.	38. 5	.....	.....	. 04	.....
10 cc. spores heated to 100° C.	38. 5	.....	.....	. 04	.....
5 cc. spores heated to 63° C.	38. 5	.....	.....	. 04	.....
10 cc. spores heated to 63° C.	38. 5	.....	.....	. 04	.....
20 cc. spores heated to 63° C.	38. 4	0. 1	0. 02	. 05	0. 01
20 PER CENT SUGAR SOLUTION					
10 cc. sterile water heated to 63° C.	73. 4	.....	.....	0. 04	.....
10 cc. sterile water heated to 100° C.	73. 4	.....	.....	. 04	.....
10 cc. spores heated to 100° C.	73. 5	.....	.....	. 04	.....
5 cc. spores heated to 63° C.	72. 7	0. 7	0. 17	. 08	0. 04
10 cc. spores heated to 63° C.	73. 1	. 3	. 07	. 18	. 14
20 cc. spores heated to 63° C.	72. 6	. 8	. 19	. 29	. 25

TABLE VIII.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Penicillium expansum*

10 PER CENT SUGAR SOLUTION

Treatment.	After three days' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 63° C.	39. 5	.....	.....	0. 04	.....
10 cc. sterile water heated to 100° C.	39. 5	.....	.....	. 04	.....
10 cc. spores heated to 100° C.	39. 5	.....	.....	. 04	.....
5 cc. spores heated to 63° C.	39. 1	0. 4	0. 10	. 05	0. 01
10 cc. spores heated to 63° C.	38. 3	1. 2	. 30	. 06	. 02
20 cc. spores heated to 63° C.	38. 1	1. 4	. 35	. 05	. 01
20 PER CENT SUGAR SOLUTION					
10 cc. sterile water heated to 63° C.	74. 4	.....	.....	0. 04	.....
10 cc. sterile water heated to 100° C.	74. 4	.....	.....	. 04	.....
10 cc. spores heated to 100° C.	74. 4	.....	.....	. 04	.....
5 cc. spores heated to 63° C.	73. 6	0. 8	0. 19	. 14	0. 10
10 cc. spores heated to 63° C.	72. 6	1. 8	. 44	. 18	. 14
20 cc. spores heated to 63° C.	72. 7	1. 7	. 42	. 32	. 28

The same procedure was presently followed, using the spores of *Aspergillus flavus* as an inoculum to the extent of 300,000 per cubic centimeter. The results are to be found in Table IX.

TABLE IX.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Aspergillus flavus*

Treatment.	After three hours' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 PER CENT SUGAR SOLUTION					
10 cc. sterile water heated to 63° C.	39.5	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	39.5	.....	.....	.04	.....
10 cc. spores heated to 100° C.	39.5	.....	.....	.04	.....
10 cc. spores heated to 63° C.	39.5	.....	.....	.08	0.04
10 cc. spores heated to 63° C.	39.5	.....	.....	.14	.10
10 cc. spores heated to 63° C.	40.1	.....	.....	.07	.07
20 PER CENT SUGAR SOLUTION					
10 cc. sterile water heated to 63° C.	72.0	.....	.....	0.04	.....
10 cc. sterile water heated to 100° C.	72.0	.....	.....	.04	.....
10 cc. spores heated to 100° C.	72.1	.....	.....	.04	.....
5 cc. spores heated to 63° C.	71.8	0.2	0.04	.10	0.06
10 cc. spores heated to 63° C.	72.2	.....	.....	.16	.12
20 cc. spores heated to 63° C.	71.9	.1	.02	.20	.16

It will be seen that there was a slight gain in reducing sugars in both 10 and 20 per cent sugar solutions inoculated with spores of *Aspergillus flavus* heated to 63° C. The polarization values are all within experimental error. Upon longer incubation only negligible differences were obtained, which makes it superfluous to record the results. Suffice it to say that one may conclude from the above data that the spores of *Aspergillus flavus* contain very little, if any, invertase.

It is of special interest to consider this problem with regard to the spores *Aspergillus sydowi*, because it has been shown by us[1] that this mold not only occurs with greatest frequency on all types of sugar investigated but furthermore has the greatest deteriorative power of all of the molds isolated. The inoculum used in the present instance contained 600,000 spores per cubic centimeter. The results obtained with it are recorded in Table X.

After three hours' incubation there was a loss of sucrose in both 10 and 20 per cent sugar solutions with 10 and 20 cc. of inoculum. This was further emphasized at the end of two days, when losses of 1.5 and 2 per cent sucrose are to be noted with 20 cc. of inoculum in the 10 and 20 per cent sugar solutions, respectively. The striking fact encountered was that

[1] KOPELOFF, Nicholas, and KOPELOFF, Lillian. OP CIT.

all the cultures inoculated with spores heated to 63° C. were a bluish-gray color, and it appeared that there was considerable matter in suspension. This proved to be a gum, which was precipitated by five volumes of 95 per cent alcohol and came down most abundantly in a strongly alkaline solution. The amount of gum present increased in proportion to an increase in inoculum. It was important to determine whether or not the presence of this gum affected the polarization of the foregoing solutions. Consequently the gum was precipitated from each of two flasks of the 10 and 20 per cent sugar solutions, and the sugar was filtered. The polarization of this filtrate was identical with that of the unfiltered solution, proving that the gum when present in such amount did not influence the polarization value beyond the experimental error of the method employed. The gum was found to exert no influence on Fehling's solution. Further data regarding the properties and nature of this gum are now being obtained and will soon be available for publication.

TABLE X.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Aspergillus sydowi*

Treatment.	After three hours.			After two days.			Filtrate after alcohol precipitation.		
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Polarization	Decrease in polarization.	Decrease in sucrose.	Polarization.	Decrease in polarization.	Decrease in sucrose.
			Per ct.			*Per ct.*			*Per ct.*
10 PER CENT SUGAR SOLUTION									
10 cc. sterile water heated to 63° C.	45.8	.....	.....	45.6	.....	.....	44.5	.....	.....
10 cc. sterile water heated to 100° C.	46.0	.....	.....	46.3	.....	.....	44.5	.....	.....
10 cc spores heated to 100° C.	45.5	0.3	0.07	45.6	.....	.....	44.5	.....	.....
5 cc. spores heated to 63° C.	46.1	.....	.....	44.5	1.1	0.27	42.8	1.7	0.43
10 cc. spores heated to 63° C.	45.5	.3	.07	41.6	4.0	1.00	40.3	4.2	1.04
20 cc. spores heated to 63° C.	44.2	1.6	.40	39.7	5.9	1.47	37.4	7.1	1.77
20 PER CENT SUGAR SOLUTION									
10 cc. sterile water heated to 63° C.	72.2	.....	.....	72.8	.....	.....	70.5	.....	.....
10 cc. sterile water heated to 100° C.	72.2	.....	.....	72.6	0.2	0.05	70.5	.....	.....
10 cc. spores heated to 100° C.	72.2	.....	.....	72.5	.3	.07	70.5	.....	.....
5 cc. spores heated to 63° C.	72.9	.....	.....	70.9	1.9	.47	68.3	2.2	0.55
10 cc. spores heated to 63° C.	72.1	0.1	0.02	69.0	3.8	.94	67.6	2.9	.72
20 cc. spores heated to 63° C.	70.4	1.8	.44	64.0	8.2	2.05	62.1	8.4	2.10

It was considered advisable to precipitate the gum, filter, and again polarize the solutions after two days' incubation. These values are shown in the last columns of Table X.

The same general relationships as noted in the solutions containing gum were again established. The fact that some evaporation occurred and that these polarizations were made with a 100-mm. tube accounts for the slightly higher values obtained.

After 2½ days' incubation the solutions were again polarized and reducing sugars determined, with the results recorded in Table XI.

TABLE XI.—*Analyses of 10 and 20 per cent sugar solutions inoculated with spores of Aspergillus sydowi*

10 PER CENT SUGAR SOLUTION

Treatment.	After 2½ days' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 cc. sterile water heated to 63° C.	46. 1	.....	.....	0. 12	.....
10 cc. sterile water heated to 100° C.	46. 2	.....	.....	. 12	.....
10 cc. spores heated to 100° C.	45. 8	0. 3	0. 07	. 12	.....
5 cc. spores heated to 63° C.	44. 4	1. 7	. 42	. 57	0. 45
10 cc. spores heated to 63° C.	40. 9	5. 2	1. 30	. 90	. 78
20 cc. spores heated to 63° C.	38. 6	7. 5	1. 87	1. 24	1. 12

20 PER CENT SUGAR SOLUTION

10 cc. sterile water heated to 63° C.	73. 0	.....	.....	0. 44	.....
10 cc. sterile water heated to 100° C.	73. 0	.....	.....	0. 44	.....
10 cc. spores heated to 100° C.	72. 9	0. 1	0. 02	. 52	0. 08
5 cc. spores heated to 63° C.	71. 0	2. 0	. 50	. 81	. 37
10 cc. spores heatedt o 63° C.	68. 2	4. 8	1. 19	1. 44	1. 00
20 cc. spores heated to 63° C.	63. 5	9. 5	2. 37	1. 80	1. 45

It is at once apparent that there is a striking decrease in sucrose accompanied by an increase in reducing sugars with an increase in the number of spores used for inoculation, and that as with the spores of *Aspergillus niger* the invertase activity is manifest to a greater degree in the 20 per cent than in the 10 per cent sugar solutions.

In order to identify the invertase and gum-forming enzym with the spores of *Aspergillus sydowi* alone, each solution was examined microscopically and plated out in the usual manner on Kopeloff's agar.[1] No contaminating bacteria or other microorganisms were to be found.

Having established that the spores of some molds contain invertase and gum-forming enzym, it is essential to define the limits of concentration under which these enzyms may operate. Consequently, a 70

[1] KOPELOFF, Nicholas, and KOPELOFF, Lillian. OP. CIT.

per cent (by weight) sugar solution was prepared and diluted to concentrations of 10, 20, 30, 40, 50, and 60 per cent and sterilized in the usual manner. All flasks were inoculated with 10 cc. of spores of *Aspergillus sydowi* containing 120,000 spores per cubic centimeter. The uninoculated flasks received 10 cc. of sterile water heated to 63° C. After 2¼ days' incubation the solutions were analyzed as shown in Table XII.

TABLE XII.—*Analyses of sugar solutions of increasing concentration inoculated with spores of Aspergillus sydowi*

Treatment.	After 2¼ days' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 per cent solution, uninoculated	24.0	.....	.....	0.15	.....
10 per cent solution, inoculated with spores at 100° C.	24.0	.....	.....	.15	.....
10 per cent solution, inoculated with spores at 63° C.	22.8	1.2	0.30	.42	0.27
20 per cent solution, uninoculated	48.0	.....	.....	.30	.....
20 per cent solution, inoculated with spores at 63° C.	46.4	1.6	.40	.73	.43
30 per cent solution, uninoculated	72.0	.....	.....	.40	.....
30 per cent solution, inoculated with with spores at 63° C.	71.7	.3	.07	.43	.03
40 per cent solution, uninoculated	96.0	.....	.....	.51	.....
40 per cent solution, inoculated with spores at 63° C.	96.0	.....	.....	.50	.....
50 per cent solution, uninoculated	120.0	.....	.....	.60	.....
50 per cent solution, inoculated with spores at 63° C.	120.0	.....	.....	.60	.....
60 per cent solution, uninoculated	144.0	.....	.....	.80	.....
60 per cent solution, inoculated with spores at 63° C.	143.9	.....	.....	.80	.....
70 per cent solution, uninoculated	168.0	.....	.....	.80	.....
70 per cent solution, inoculated with spores at 63° C.	168.3	.....	.....	.80	.....

It will be seen that inversion occurred in the solutions of 10, 20, and 30 per cent concentration but did not take place in higher concentrations. The formation of gum already described was also found at the former concentrations. Again it will be noted that there was greater inversion with a 20 per cent than with a 10 per cent solution. Unfortunately the inoculation was too meager to produce as large a change as might be desired, and it is altogether likely that an increased inoculation would be more active. Thus, for the inoculum employed, the limit of concentration appears to be between 30 and 40 per cent by weight, which, recalculated to actual percentage of sucrose in the solution, is between 18 and 24 per cent.

The same experiment was repeated, using the spores of *Aspergillus niger* to the extent of 400,000 per cubic centimeter. The result is recorded in Table XIII.

TABLE XIII.—*Analyses of sugar solutions of increasing concentration inoculated with spores of Aspergillus niger*

Treatment.	After 2½ days' incubation.				
	Polarization.	Decrease in polarization.	Decrease in sucrose.	Reducing sugars.	Increase in reducing sugars.
			Per cent.	*Per cent.*	*Per cent.*
10 per cent solution, uninoculated	24.0	.....	.....	0.14	.....
10 per cent solution, inoculated with spores at 100° C.	24.0	.....	.....	.14	.....
10 per cent solution, inoculated with spores at 63° C.	22.9	1.1	0.27	.32	0.18
20 per cent solution, uninoculated	48.0	.....	.....	.20	.....
20 per cent solution, inoculated with spores at 63° C.	47.4	.6	.15	.29	.09
30 per cent solution, uninoculated	72.0	.....	.....	.33	.....
30 per cent solution, inoculated with spores at 63° C.	71.6	.4	.10	.35	.02
40 per cent solution, uninoculated	96.0	.....	.....	.45	.....
40 per cent solution, inoculated with spores at 63° C.	96.0	.....	.....	.45	.....
50 per cent solution, uninoculated	120.0	.....	.....	.56	.....
50 per cent solution, inoculated with spores at 63° C.	120.0	.....	.....	.57	.....
60 per cent solution, uninoculated	144.0	.....	.....	.70	.....
60 per cent solution, inoculated with spores at 63° C.	144.0	.....	.....	.73	.....
70 per cent solution, uninoculated	168.0	.....	.....	.80	.....
70 per cent solution, inoculated with spores at 63° C.	168.0	.....	.....	.81	.....

It will be observed that inversion occurred to a slight extent in 10, 20, and 30 per cent concentrations but not beyond this point. This corroborates the results previously obtained with the spores of *Aspergillus sydowi* and establishes the upper limit of concentration for the invertase activity of these spores. When recalculated it is found to be between 18 and 24 per cent actual sucrose in the solution.

The query, therefore, which was considered as a basis for this investigation, Do mold spores contain enzyms? has been answered in the affirmative by virtue of the evidence advanced.

It is irrelevant at the present time to do more than indicate the significance of the industrial applications of this biological principle. We have already mentioned that it plays a considerable rôle in the deterioration of sugar by materially affecting the factor of safety rule. It may likewise explain certain transformations in the soil which occur as a result of mold activity where mycelia are not found in great abundance. Thus the universal distribution of molds and their activities will undoubtedly suggest many further developments of this phenomenon, some of which are at present under investigation in this laboratory.

SUMMARY

(1) The spores of *Aspergillus niger*, *Aspergillus sydowi*, and to a lesser extent *Penicillium expansum* and *Aspergillus flavus*, heated to 63° C. for 30 minutes and shaken with sterile sand, caused a decrease in polarization and an increase in reducing sugars in a 10 per cent sterile sugar solution in 3 hours and continued to cause the same changes throughout the 4-day incubation at 45°. Increased activity of a corroborative nature was obtained with 20 per cent sugar solutions. An increase in the number of spores caused an increase in enzymic activity.

(2) The fact that neither spores heated to 100° C. nor an inoculation with sterile distilled water caused any change, indicated the activity mentioned above to be enzymic in nature.

(3) The enzym present exhibited activities indentical with ivertase, consequently the spores of *Aspergillus niger*, *Aspergillus sydowi*, *Penicillium expansum*, and *Aspergillus flavus* contain invertase.

(4) The spores of blue aspergillus contained a gum-forming enzym which paralleled invertase activity.

(5) The limit of concentration of 100,000 to 400,000 mold spores per cubic centimeter for both the invertase activity and the formation of gum was found to be between 18 and 24 per cent actual sucrose.

(6) Among the practical applications of this phenomenon the deterioration of manufactured cane sugar and certain transformations in the soil are especially significant.

NATURE AND CONTROL OF APPLE-SCALD

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NATURE OF APPLE-SCALD

EFFECT OF REMOVAL FROM STORAGE

It is a quite generally accepted idea that apple-scald is due to the warming up of the fruit after it has been removed from cold storage. This idea comes from an erroneous interpretation of very familiar facts (*2*).[1] It is true that apples do not show scald while held continuously in storage at 0°C. (32° F.) and that they seldom show it under commercial cold-storage conditions where the air must from necessity sometimes vary slightly from any desired temperature. It is also true that apples that have been stored for several months in tight packages or closed rooms may show very bad scald after a few days' exposure to warm air. Under such circumstances, it is natural to conclude that the warming up of the fruit is the cause of the scald; but the facts of the case are that the apples are already potentially scalded, and the higher temperatures merely allow the death processes of the apple tissue to be carried out. The real cause of the disease is to be found in the conditions of transportation and storage to which the fruit is subjected during the first few weeks after it is removed from the tree. Healthy apples do not develop scald upon removal from cold storage, even when transferred at once to living-room temperatures.

RELATION OF THE COMPOSITION OF THE STORAGE AIR TO APPLE-SCALD

The fact that outside air produces such serious results on potentially scalded apples has led to a belief that apples should be kept away from fresh air and air currents as much as possible at all times; but carefully controlled storage experiments have shown conclusively that when the fruit is stored in open packages, scald can be entirely prevented by thorough stirring of the storage air. The question naturally arises as to the nature of the harmful substances that are carried away from the fruit by this air movement.

HUMIDITY.—Many storage men hold the opinion that excessive moisture may bring about apple-scald. The writers have made a number of carefully controlled experiments with apples held under different humidities and, as reported later, have also followed up the moisture conditions

[1] Reference is made by number (italic) to "Literature cited," p. 240.

in commercial cold storage. The results indicate that when moisture remains condensed in drops on the fruit there may be a slight increase in the development of scald, but apparently because of the more restricted aeration of the apple tissue rather than from any harmful effect of the water itself. Apples stored in air that was saturated with moisture but constantly stirred have not developed scald, while similar apples in dry, stagnant air have become badly scalded. Apples have been exposed to outside air by throwing a cold-storage room open freely when the temperature would allow, and by rolling the barrels out of the storage room for one or two days each week; but scald has been reduced rather than increased by the treatment. (See p. 223.) Apples that have been picked wet or have had water poured over them after they were in the barrel have developed no more scald than others picked and packed when they were dry. The writers have found no evidence that excessive humidity plays any important part in the development of scald either under experimental or commercial storage conditions.

Oxygen.—The apple is a breathing organism, and under conditions of restricted aeration the percentage of oxygen in the air surrounding the fruit is reduced below normal. The question naturally arises as to whether this change in air composition has any influence upon the development of scald. To test this point, apples were stored in air which had the percentage of oxygen reduced from 21 to 6.9, and others in air which had the percentage of oxygen increased to 31.5. The amount of scald developed in each lot was compared with the amount on apples held in air having the normal percentage of oxygen. The reduced oxygen supply resulted in no increase in the amount of scald, and the increased oxygen supply gave no significant decrease in scald. In other experiments, Grimes apples were held in 100 per cent oxygen at 20° C. for four days and then removed from the oxygen and placed in moist chambers in normal air, part of the apples being then stored at 15° and part at 2.5°. Other apples were exposed to the same temperatures but were not given the preliminary oxygen treatment. Notes were taken at various times on the amount of scald, but no difference of any kind developed between the apples that had been first stored in oxygen and those that had not. The results are strikingly different from those reported later from similar experiments with carbon dioxid. (See p. 213.) The results of the various experiments seemed to prove conclusively that the small variations in the oxygen content that ordinarily occur in the storage air are not matters of importance in determining the development of scald.

Ozone.—Although an increased oxygen supply resulted in little or no decrease in the amount of scald, it seemed possible that a more powerful oxidizing agent might give different results. So in the fall of 1918 both laboratory and commercial cold-storage experiments were made with ozone.

In the laboratory tests a small but powerful ozone machine was used. In the preliminary experiments the ozonated air was used in its full strength as it came from the machine; but it injured the apples, soon producing brown dead spots at the lenticels. In the final experiments the air from the ozone machine was reduced to half strength by mixing with an equal volume of normal air. The apples used in the tests were Grimes from Vienna, Va. They were picked August 21 and the experiments started the following day. The fruit was stored in 8-liter jars that were fitted with 2-hole stoppers. Once every week ozonated air was drawn through these jars, the incoming current being freed at the bottom of the jars and the outgoing current taken from the top. The process was continued for 5 minutes and the jar then tightly corked and allowed to stand closed for the next 24 hours, after which the ⅝-inch stopper was removed and the jar left as a moist chamber for the remainder of the week. At the end of the ozone treatment the air from the exit tubes had a strong smell of ozone, but after 24 hours no ozone odor could be detected in the air of the jars. Part of the apples were stored at 15° and part at 0° C. In each experiment, control jars of apples were maintained that were identical with the others in every respect, except that in the weekly treatment normal air was drawn through instead of the ozonated air. Notes were taken at various times on the amount of scald and on the quality of the fruit, but no contrast of any kind was found between the apples that were treated with ozone and those that were not.

In the commercial cold-storage experiments, the ozone was obtained from a large 12-cylinder machine of the type most commonly used in egg storage rooms. The machine was operated from six to eight hours a day and from five to seven days a week. Six barrels of Grimes apples and six barrels of York Imperial were stored within a few feet of the machine, while similar lots were held as controls in another storage room. In each test, half of the apples were in ventilated barrels and half in barrels of the usual commercial type. The final notes on the Grimes apples were taken December 20 and the final notes on the York Imperial on January 18. Both varieties had scalded badly; but there was no contrast, in either the ventilated or unventilated barrels, between the fruit that had been exposed to ozonated air and that which had not. The results give little promise of scald prevention by increased oxidation.

CARBON DIOXID.—Carbon dioxid is the gas produced in greatest quantity by storage fruit and is therefore the one that might most naturally be expected to produce harmful results. Experiments reported in an earlier paper (*2*), however, have shown that apples stored continuously in atmospheres having percentages of carbon dioxid similar to those in commercial storage, or even considerably exceeding them,

have shown no sign of injury and have developed less scald than similar apples held in air that was free from carbon dioxid. It was also found that apples could be made less susceptible to scald by storing them for a few days in an atmosphere composed entirely of carbon dioxid but that this treatment sometimes gave the apples a disagreeable alcoholic taste. In order to get further data as to the carbon-dioxid endurance of the apple, these latter experiments were repeated in the fall of 1918 with the period of storage in carbon dioxid shortened. The results are given in Table I. Lots A, B, and C were Grimes from Vienna, Va. They were picked August 20 and the experiment started the following day. The apples in lot D were Grimes from Wenatchee, Wash. They were shipped to Washington, D. C., in a pony refrigerator and the experiment started September 20. Lot E was Yellow Newtown apples from Winchester, Va. They were in cold storage until December 19, the date of starting the experiment. In all the tests the apples receiving prestorage treatment with carbon dioxid were removed from this gas at the end of the given number of days and stored in moist chambers in normal air. Thereafter they had the same air and moisture conditions as the controls had from the beginning of the experiment.

TABLE I.—*Effect of prestorage treatment with carbon dioxid upon the development of apple-scald*

Lot.	Prestorage conditions.		Storage temperature.	Number of weeks in storage.	Percentage of scald.	
	Temperature.	Number of days held.			Apples held in 100 per cent carbon dioxid during prestorage period.	Apples held in normal air during prestorage period.
	°C.		°C.			
A	30	2	10	10	10	52
B	20	4	15	10	35	60
C	20	4	2½	16	4	38
D	15	2	5	13	10	50
D_1	15	6	5	13	6	.....
D_2	30	2	5	13	0	.....
E	15	6	0	15	0	15

At the end of the various experiments the apples that had been treated with carbon dioxid were slightly greener than the untreated fruit but were apparently normal in taste and general appearance. A study of the last two columns of the table will show that the apples exposed to carbon dioxid developed much less scald than those that were not, giving further evidence that carbon dioxid has no tendency to produce the disease. The writers are of the opinion that the apparently beneficial effects of the carbon dioxid treatment are due to a general checking of the skin activities of the apple rather than to any specific

favorable effect upon apple scald. The results of these and earlier experiments show, however, that it is possible to use carbon dioxid as an agency for reducing apple scald and that this can be accomplished without evident injury to the apple.

While it seems to have been conclusively proved that carbon dioxid is not responsible for the occurrence of apple scald, it does not follow that high percentages of the gas in storage air are to be looked upon with favor, for such a condition would indicate a lack of air movement and an accumulation of other gaseous products of the apple as well as of carbon dioxid.

ARTIFICIAL SCALD.—Various attempts have been made to produce apple-scald artificially or to shorten its period of development by changing the composition of the air; but, as mentioned in the discussions of humidity and carbon dioxid, these have usually met with failure. Other experiments of this sort were made with alcohols, acids, and esters. In the preliminary tests the various substances were used in full strength and were placed in close proximity to the fruit, but this led to such serious and rapid injury from the more active agents that nothing resembling scald was produced. In the later experiments dilutions were made as indicated below, and the liquids were placed in the bottom of 8-liter jars with the apples supported in the top at a distance of approximately 12 inches from the chemicals. Twenty-five cc. of the material were used in each jar. All the jars were loosely stoppered. The experiments were made at 10° C. The results reported below are based on the appearance of the apples after they had been removed from the storage condition and had stood in a warm laboratory for 24 hours.

TABLE II.—*Effects of various volatile substances on Yellow Newtown and Rome Beauty apples*

Substances used.	Effects.
Water, control	Apples stored over water developed no scald or other injury.
Ethyl alcohol	At the end of 3 weeks no scald had developed, and the apples were apparently still normal.
Acetic acid	Rome Beauty apples showed injury at the end of 24 hours, but the effects did not resemble scald.
Alcohol 60 per cent, acetic acid 40 per cent.	Rome Beauty apples had their flesh killed and browned to a depth of ¼ inch at the end of 48 hours, but the effects did not resemble scald.
Alcohol, 90 per cent, acetic acid, 10 per cent.	At the end of 7 days dead brown areas of various patterns were scattered over the skin of the apple. Many of the smaller spots were located at the lenticels. There was a clear-cut margin between the diseased and the healthy areas, and the flesh was affected to a much greater depth than it would be by scald. The brown spots were as common on the highly colored portions of the skin as on the poorly colored ones. There was but slight resemblance to typical scald.
Formic acid 100 per cent	No scald or other injury after 3 weeks.

TABLE II.—*Effect of various volatile substances on Yellow Newtown and Rome Beauty apples*—Continued

Substances used.	Effects.
Alcohol 80 per cent, ethyl acetate 20 per cent.	After 3 days' exposure to the vapors, Rome Beauty apples had a brown, cooked appearance, the red portions of the apples, however, being much less affected than the green portions.
Alcohol 90 per cent, ethyl acetate 10 per cent.	After 7 days, Rome Beauty and Yellow Newtown apples had the appearance of being typically scalded. The scalded areas occurred only on the green side of the fruit and shaded off in severity as the blush areas were approached. After standing in a warm room for 4 days the browning had spread into the flesh of the apple rather more rapidly than is usual with scald, but aside from this the diseased condition was typical of apple-scald.
Alcohol 80 per cent, amyl acetate 20 per cent.	Only Rome Beauty apples were tested. The results were practically the same as with 10 per cent ethyl acetate, a quite typical scald being produced.
Alcohol 90 per cent, amyl acetate 10 per cent.	Experiments were made with both Rome Beauty and Yellow Newtown apples. The former were removed at the end of 3 days and the latter at the end of 7 days. The results were similar to those reported for 10 per cent ethyl acetate, but the apples were even more typically scalded. The browned areas coincided in the most exact manner with the skin areas naturally susceptible to scald.
Ethyl malate 10 per cent.....	Only about 10 cc. of the liquid were used, and the apples were placed in a smaller jar than those mentioned above. No scald or other injury had been produced at the end of 3 weeks.
Ethyl butyrate.....	Experiments somewhat similar to those described above gave results very much like those obtained with ethyl acetate and amyl acetate.

The results reported in Table II show that it is possible to produce an apparently typical apple-scald within a few days by exposing the fruit to the vapors of certain dilute esters. The fact that the disease can be thus produced artificially when connected with the additional fact that the apples themselves are known to give off esters or related gases makes it seem probable that these substances play an important part in the development of scald as it occurs in commercial storage.

GAS ABSORBENTS AS SCALD PREVENTIVES

In an earlier paper (5) experiments were reported showing that scald can be practically prevented by wrapping the apples in paper that has been infiltrated with fat or oil. Other experiments are reported later in the present paper (see p. 233) that give full confirmation of these results and also make it clear that the beneficial effects of the wrappers are not due to modifications in the moisture or the carbon-dioxid content of the air surrounding the apple. It is well known that fats and oils have a great absorbing power for esters and other odorous gases, and because of this property they are used commercially in the extraction of perfumery. Cow butter takes up odors so readily that it is usually rendered unpal-

atable if held in storage with other food products. In view of these facts there seems to be little doubt that the beneficial effects of the fats and oils in the wrappers are due to the absorption of esters or similar products thrown off by the apple. The hypothesis is given further support by the fact that the fats and oils which are known to have a great absorbing power for gases give more complete control of scald than paraffin and similar waxes that are generally recognized as being more inactive toward these gases, and also by the results in the experiments reported above in which scald was produced artificially by exposing apples to various esters.

It is generally recognized in the apple trade that greasy, waxy apples do not scald as soon or as badly as the others. The above hypothesis offers a possible explanation for this fact, the wax of the apple, like that in the wrapper, apparently serving as an absorbent for the harmful gases.

TISSUES AFFECTED BY APPLE-SCALD

Scald is typically a skin disease of the apple. In the early and more typical stages of the trouble, only the five or six surface layers of cells that form the color-bearing tissue of the apple are affected. With long continued unfavorable conditions the apple tissue may become dead, brown, and rotlike to a depth of ⅛ to ¼ inch, and occasionally the disease spreads practically to the core. Reference is made here to the scald itself; but with the death of the protective skin layer, various rot organisms have free access to the softer tissues beneath and usually play an important part in hastening the destruction of the apple. Not all portions of the skin are equally susceptible to scald. The highly colored areas of red apples are affected only in the most extreme cases of scald. Often when the poorly colored areas are badly and deeply scalded, the diseased condition will shade off into a mere brown tint of the skin as the margin of the blush area is approached. The chemical changes that occur in the reddening of the fruit apparently produce a skin condition that is highly resistant to scald.

The statement is quite generally current that apples that still show the leaf green are very much more susceptible to scald than those which have become slightly yellowed. As a rough statement of the facts, this may be approximately true. The observations of the writers, however, indicate that while green apples, in general, are more susceptible to scald than ripe ones, those still having the leaf green are very much less susceptible than those that have just begun to turn yellow, and often less susceptible than those in which the ground color has become a deep yellow. They have also observed that while green apples may finally become more severely scalded than riper ones, the latter usually scald first if they develop the disease at all. In spite of these qualifications, it is still true that scald can be greatly reduced and delayed by leaving the apples on the tree till well matured.

INFLUENCE OF ORCHARD CONDITIONS

It is generally admitted that the susceptibility of apples to scald probably varies with orchard conditions, but little experimental data has ever been published on the subject. In the fall of 1918 apples were secured from various soil and orchard conditions at Wenatchee, Wash., for comparative storage tests on this point. In one test, Grimes apples from lightly irrigated trees growing in heavy clay soil were compared with similar apples from lightly irrigated trees on alluvial sand receiving a spring application of 10 pounds of sodium nitrate per tree. The apples were stored in the usual box packages in commercial cold storage. They were removed to a temperature of 15° C. (59° F.) on February 6 and notes taken February 15. The apples from the heavy clay soil had 35 per cent of scald and those from the heavily fertilized sandy soil 60 per cent of scald.

Experiments were conducted also with apples from a Grimes orchard in which irrigation experiments were being made. The orchard was in alfalfa and the soil of the various plots was quite uniform. The contrasts in irrigation were started the first of July and maintained for the rest of the season. With the heavily irrigated plot the soil moisture was kept at approximately 50 per cent of saturation, and with the lightly irrigated one at approximately 20 per cent of saturation, while with the third plot the soil moisture was kept at approximately 20 per cent from July 1 to August 15, and then at approximately 50 per cent the remainder of the season. The methods of irrigation, soil sampling, etc., were the same as those reported in an earlier paper (4) and will not be repeated here. The apples were picked on September 18, when mature but not overripe, and placed in storage the following day. They were removed from storage February 5 and were held at a temperature of 15° C. (59° F.) for one week before notes were taken. In experiment A one box of apples from each plot was used under each storage condition. In experiment B separate records were made for the apples of different sizes. There were from 1 to 6 pecks of each size under each storage condition. The results are given in Table III.

The apples from the plot receiving light irrigation early and heavy irrigation late developed about twice as much scald as those from the plot receiving heavy irrigation continuously and three to four times as much as those from the lightly irrigated plot.

It is particularly interesting to note that the increased amount of scald on the heavily irrigated apples was not due to their larger size, since the increase was as great on the small apples as on the large ones. It has been observed in an earlier publication (5) that large apples often scald worse than small ones. The foregoing results indicate that in the present case size is a secondary factor, the real cause of the increased scald being

some forcing agency, such as heavy irrigation, that has apparently rendered both large and small apples more susceptible to the disease.

TABLE III.—*Effect of orchard irrigation upon the development of scald in storage: Experiments with Grimes apples at Wenatchee, Wash., 1918*

Kind of storage.	Irrigation.	Percentage of scald.				
			Experiment B.			
		Experiment A.	Apples 3¼ inches and smaller.	Apples 2⅝ to 3 inches.	Apples 3 to 3¼ inches.	Apples 3¼ to 3½ inches.
Cellar	Heavy	83	92	88	90	73
	Light	22	23	21	15	No apples.
	Light followed by heavy	50	.....	.....	.....	.....
Air-cooled	Heavy	59	42	56	64	75
	Light	17	14	19	5	No apples.
	Light followed by heavy	40	.....	.....	.....	.....
Cold	Heavy	69	53	79	75	61
	Light	23	6	27	28	No apples.
	Light followed by heavy	57	.....	.....	.....	.....

RELATION OF TEMPERATURE TO THE OCCURRENCE OF APPLE-SCALD

The temperature relations of apple scald have been rather fully discussed in earlier reports (5). The rate of scald development increases with a rise in temperature; between 0° and 20° C. each rise of 5° hastens the time of scald appearance by two to six weeks, the greatest contrast occurring between 5° and 10° and the least between 15° and 20°. At 0° scald does not become evident. The apples become latently or potentially scalded but give little evidence of it until removed to a warmer temperature. At temperatures of 25° and above it has not been found possible to produce apple-scald, although other physiological troubles, such as internal breakdown, have developed all the more rapidly at these temperatures. Scald has been greatly delayed and in some cases apparently entirely prevented by bringing apples out after four or five weeks in commercial cold storage and giving them a thorough airing for 24 hours at a temperature of 22°. The hypothesis that scald is due to the accumulation of apple esters may furnish at least a partial explanation for the peculiar effects of the higher temperatures. The fruit esters are in general quite volatile, and their rate of vaporization is greatly increased by a rise in temperature. It seems possible that the slight increase in the rate of scald development in passing from 15° to 20° and the absence of the disease at 25° and 30° may be partly if not entirely due to the greater vaporization of the harmful products at these higher temperatures. It should not be overlooked,

however, that there is a marked change in the general ripening processes of the apple at the higher temperatures and that there may be much more fundamental reasons than the one suggested above for the absence of scald at these temperatures.

EXPERIMENTS IN THE CONTROL OF APPLE-SCALD

In the fall of 1918 apple storage experiments were started in Wenatchee, Wash., Winchester, Va., and Washington, D. C. In the smaller lots the apples were carefully selected from the tree, and in the larger lots they were taken as they came from the packing table. In obtaining records on the degree of scald the maximum scald that had been observed on the variety was taken as 100, and the amount of scald in a particular case was determined by its relation to this standard. Consideration was given to the area and depth of the scald as well as to the number of apples affected.

As previously mentioned (p. 211) apples may be potentially or latently scalded and yet not show it while held continuously at 0° C. (32° F.). In order to get the actual condition of the fruit as it came from storage, it was therefore held at a temperature of 20° C. (68° F.) for three days before the final notes were taken.

RELATION OF MATURITY OF FRUIT TO APPLE-SCALD

Powell and Fulton (*8*) were apparently the first to call attention to the importance of the maturity of the fruit in the control of apple-scald. Beach (*1*), Greene (*6*), Markell (*7*), Ramsey (*9*), and others have published confirmatory data. As pointed out earlier in this article (p. 217), the writers have found some definite exceptions to the rule that green fruit scalds worse than ripe, but in general their experimental data support the work of earlier investigators.

Table IV gives the results obtained with early and late pickings of Grimes, Rome Beauty, and York Imperial apples. The fruit was stored promptly in all tests. The Grimes and Rome Beauty apples were held in air-cooled cellar storage at Wenatchee, Wash. During September the average temperature of the cellar was 15° C. (59° F.), and the average humidity 75 per cent; during October the average temperature was 11° C. (51.8° F.), and the average humidity 72 per cent; and for the remainder of the storage period the average temperature was 2.5° C. (36.5° F.), and the average humidity 82 per cent. There was a daily fluctuation of from 2° to 4° C. The data given in the table were obtained after the apples had been held at 20° C. (68° F.) for nine days. The percentages in each case are practically double those recorded at the time of removal from cellar storage. One box of apples was used from each picking. The York Imperial apples were held at 0° C. (32° F.) in direct expansion commercial cold storage at Winchester, Va. Three barrels of apples were used under each condition in each test.

All the apples were practically free from scald when removed from storage. The data reported were obtained after the fruit had been held at 20° C. (68° F.) for three days.

TABLE IV.—*Relation of maturity of fruit to apple-scald*

Variety and location.	Package.	Date of picking.	Condition of fruit.	Date of note taking.	Weeks in storage.	Percentage of scald.
Grimes at Wenatchee, Wash.	Box	Sept. 7	Immature	Feb. 15	23	52
		Sept. 17	Mature commercial picking.	do	22	30
		Oct. 2	Ripe	do	19	18
Rome Beauty at Wenatchee, Wash.	do	do	Green	Mar. 15	23	20
		do	Well-colored	do	23	6
		Oct. 21	Ripe to overripe	do	21	0
York Imperial at Winchester, Va.	Commercial barrel.	Oct. 1	Rather immature	Jan. 28	17	28
		do	do	Feb. 25	21	45
		Oct. 29	Mature	do	17	35
Do	Ventilated barrel.	Oct. 1	Rather immature	Jan. 28	17	11
		do	do	Feb. 25	21	28
		Oct. 29	Mature	do	17	0

In all tests the early picked fruit developed more scald than the late picked. A study of the results on York Imperial might indicate that this was largely due to the fact that the early picked apples had been in storage longer. To get a fair test of the relative susceptibility of the two pickings to scald, the February 25 data on the October picking should be compared with the January 28 data on the October 1 picking, thus giving an equal storage period (17 weeks) for each lot. This method of comparison greatly reduces the contrast between the early picked and the late picked fruit, but the latter still maintains a superiority in scald resistance. The great difficulty of scald control by means of maturity lies in the fact that it is often impracticable to leave the fruit on the tree late enough to secure the desired results.

AERATION AS A PREVENTIVE FOR APPLE-SCALD

It has been proved by carefully controlled experiments that apple-scald can be completely prevented by giving the fruit sufficient aeration. This is readily accomplished with small lots of fruit in experimental storage, and the following experiments indicate that the principle can be used to advantage under commercial storage conditions.

AERATION IN DELAYED STORAGE.

There is no period in the storage life of the apple when aeration is so important as in the first week or two after the fruit is removed from the tree, especially in cases where it is impossible to hold it at low temperatures. Table V gives the results of several experiments in delayed storage. Three barrels or three boxes of fruit were used under each condition of each test. They were all held in commercial cold storage

but were removed to a temperature of 20° C. (68° F.) and held for three days before the final notes were taken. The apples used were as follows:

A, Grimes from Franklin, Va.; picked August 30; final notes taken December 20.

B, Rome Beauty from Franklin, Va.; picked September 27; final notes taken January 28.

C, York Imperial from Greenwood, Va.; picked October 10; final notes taken January 31.

D, Grimes from Winchester, Va.; picked September 10; final notes taken January 16.

E, Stayman Winesap from Winchester, Va.; picked September 25; final notes taken January 22.

F, York Imperial from Winchester, Va.; picked October 1; final notes taken February 11.

G, Rome Beauty from Wenatchee, Wash.; picked October 2; final notes taken March 25.

TABLE V.—*Effect of delayed storage and of aeration during delay*

Variety.	Treatment.	Percentage of scald.		
		Commercial barrel.	Ventilated barrel.	Box.
A, Grimes.	Immediate storage	35	30	.....
	Delayed 10 days in open packing shed	12	3	.....
	Delayed 10 days in sun in boxes	12	.....	.....
B, Rome Beauty.	Immediate storage	28	20	.....
	Delayed 10 days in warm laboratory, temperature 21.1° to 23.9° C. (70° to 75° F.).	80	0	.....
C, York Imperial.	In transit, by express, 3 days	20	8	.....
	In transit, by freight, 15 days	60	17	.....
D, Grimes.	Immediate storage	58	12	.....
	Delayed 9 days in closed packing shed, temperature 15.5° to 23.9° C. (60° to 75° F.).	47	12	.....
E, Stayman Winesap.	Immediate storage	50	16	.....
	Delayed 6 days in hall of cold-storage plant, temperature 10° to 12.8° C. (50° to 55° F.).	70	12	.....
	As above but delayed 10 days	70	15	.....
F, York Imperial.	Immediate storage	35	25	.....
	Delayed 8 days in hall of cold-storage plant, temperature 12.8° to 15.5° C. (55° to 60° F.).	50	30	.....
	As above but delayed 15 days	65	35	.....
G, Rome Beauty.	Immediate storage	.....	.....	12
	Delayed 9 days in the open, in the shade, temperature 5.6° to 15° C. (42° to 59° F.).	.....	.....	0
	Delayed 9 days in a closed room, temperature 7.2° to 12.8° C. (45° to 55° F.).	.....	.....	7

If a study is made of the results in the first column, it will be seen that in four out of six tests scald was greatly increased by delayed storage, and in the other two (A and D) it was quite definitely decreased. The tests in which there was a decrease are those in which the apples received the greatest amount of aeration during the delay. With the

ventilated barrels, scald was greatly decreased by delay in two of the tests, decidedly increased in one, and apparently but little affected in the others. With the boxes, scald was slightly decreased by delay in a closed room and entirely prevented by delay in the open. The most striking feature of the table, however, is seen when the scald in the delayed, ventilated barrels is compared with that in the immediate storage, commercial barrels. The good effects of the more open package have far more than offset any bad effects from the delay, and have resulted in reducing the scald to about one-fourth of that on the immediately stored fruit in the unventilated or commercial barrel. Delayed storage may evidently be either favorable or unfavorable to the development of scald, depending upon the conditions under which the fruit is held. If it is possible to give good aeration during the delay, the results may be distinctly beneficial to the fruit, especially if it is rather immature; but as is shown in Table V, delay in closed rooms or in unrefrigerated cars is likely to result in the development of serious scald later in storage.

TEMPERATURE CHANGES AS A MEANS OF AERATION

It is generally believed that changes in the temperature of the fruit or the storage room are likely to produce serious results. The experiments reported in Tables VI, VII, and VIII indicate that so far as apple-scald is concerned, temperature changes may sometimes prove beneficial.

The apples used in Table VI were Grimes from Vienna, Va. They were stored September 3, and notes were taken December 20. Two barrels of apples were used under each condition. The laboratory to which part of the apples were removed stood at a temperature of 20° C. (68° F.), and the apples were held there for 24 hours at a time. The hall into which other apples were rolled had an open window but was protected from outside winds. The temperature was from 2½° to 5° C. (4½° to 9° F.) warmer than that of the storage room. The apples were left in the hall for about 24 hours at a time.

In the experiment reported in Table VII the apples were from Wenatchee, Wash. The Rome Beauty apples were stored October 2 and the Stayman Winesap October 12. The notes on both were taken March 25. The storage room stood at 0° C. (32° F.). The engine room to which part of the apples were moved had a temperature of 14.4° C. (58° F.) during the time of the first airing, and a temperature of 12.8° C. (55° F.) during the second airing. The average temperature of the outside air during the first airing was 7.8° C. (46° F.), and at the time of the second airing 8.8° C. (48° F.). One box of apples was used under each condition.

In the experiment reported in Table VIII the apples were from Winchester, Va. The Arkansas apples were stored October 28, and the notes taken February 3; the Stayman Winesap stored September 25, and the

notes taken January 23; the York Imperial stored October 1, and the notes taken February 11; the Yellow Newtown stored October 25, and the notes taken April 7. Three barrels of each variety were left continuously in storage and three rolled into the hall for a 24-hour period once each week during the first 2½ months of storage. The temperature of the storage room stood at 0° C. (32° F.) or slightly above; during October the hall had a temperature of 7° to 10° C. (44.6° to 50° F.), and in the later months a day temperature of about 5° C. (41° F.) and a night temperature of approximately 0° C. (32° F.) The hall doors were kept open, giving free circulation of outside air. Resistance thermometer bulbs were forced into apples in the center of the barrels and temperature readings taken when the apples were removed to the hall and again when they were returned to storage. The temperature of the fruit was never raised more than 1° C. (1.8° F.) by exposure to the hall temperature for 24 hours. In all the different tests the apples were held at 20° C. (68° F.) for three days before the notes were taken.

TABLE VI.—*Effect of temperature changes upon apple-scald: Experiment at Washington, D. C.*

Lot No.		Percentage of scald.	
		Grimes in commercial barrel.	Grimes in ventilated barrel.
1	In cold storage continuously 16 weeks	35	30
2	As in 1 but at 20° C. (68° F.) 1 day at the end of 6 weeks' storage	31	18
3	As in 1 but at 20° C. (68° F.) 1 day each at the end of 6 and 11 weeks' storage	25	5
4	As in 1 but at 20° C. (68° F.) 1 day at the end of 11 weeks' storage	38	35
5	As in 1 but in cold-storage hall 1 day each at the end of 5, 7, and 11 weeks		10
6	As in 1 but in cold-storage hall 1 day each at the end of 7 and 11 weeks	35	5
7	As in 1 but in cold-storage hall 1 day at the end of 7 weeks	28	12
8	As in 1 but in cold-storage hall 1 day at the end of 11 weeks	35	23

TABLE VII.—*Effect of temperature changes upon apple-scald: Experiment at Wenatchee, Wash.*

Lot No.		Percentage of scald in boxes.	
		Rome Beauty.	Stayman Winesap.
1	In cold storage continuously	11	12
2	In engine room for 6 hours on Nov. 7 and again for 6 hours on Dec. 2	14	8
3	As in 2 but removed to the open air for the same 6-hour periods	9	13

TABLE VIII.—*Effect of temperature changes upon apple-scald: Experiment at Winchester, Va.*

Lot No.	Treatment.	Percentage of scald in commercial barrels.			
		Arkansas.	Stayman Winesap.	York Imperial.	Yellow Newtown.
1	In cold storage continuously	50	67	45	10
2	Moved from cold storage to storage hall 1 day each week during the first 2½ months of storage	30	34	45	12

No harmful effects of any kind were found to result from the exposure of the apples to outside air. A study of the tables shows that scald was either not affected or else was reduced by the treatment, the results apparently depending upon the amount of aeration the apples received while out of storage. In the experiment reported in Table VI, where the commercial barrels were removed to rather poorly ventilated rooms 1 to 3 times, the treatment had practically no effect upon scald, but in the experiment reported in Table VIII, where similar barrels were removed to a well-ventilated hall 8 to 10 times, scald was considerably reduced. While the aeration reported in Table VI was apparently too slight to affect the apples in the commercial barrels, the same treatment resulted in a decided reduction of scald in the ventilated barrels, the difference apparently being due to the better aeration secured by the more open package. It is interesting to note in Table VI that while the aerations given at the end of the seventh week of storage decidedly decrease scald, those at the end of the eleventh week had but little effect upon the disease. This is in agreement with data reported in an earlier publication (*5*), indicating that with Grimes apples aerations must be made during the first 8 or 9 weeks of storage in order to have any beneficial effect upon scald.

The barrels removed from the storage rooms were exposed to more breezes than those that remained, but the aeration received by the apples which were moved was doubtless greatly increased by the air currents set up as a result of the difference between the temperature of the fruit and that of the outside air.

AIR-COOLED STORAGE

Experiments were made to determine the comparative development of apple-scald in air-cooled and cold-storage plants. The results are given in Tables IX and X. The apples used in the experiment recorded in Table IX were from Winchester, Va. Three barrels of each variety were used under each condition. The Arkansas apples were stored October 18, and the final notes taken February 3; the Yellow Newtown stored October 25,

and the final notes taken April 7; and the York Imperial stored October 29, and the final notes taken February 20. All the apples were in direct expansion cold storage from the time of storing till October 31 and were therefore well cooled before being placed in the air-cooled storage. The direct expansion storage house was located at Winchester, Va., and the air-cooled plant at Gerrardstown, W. Va. Hygrothermograph records were kept for both plants throughout the storage season. In the direct expansion rooms, the temperature was held at 0° C. (32° F.) or slightly above; and the relative humidity ranged from 65 to 90 per cent, standing between 75 and 80 per cent during most of the storage season. In the air-cooled plant, the temperature ranged from 5° to 15° C. (41° to 59° F.) during the period from October 31 to November 20 and throughout the remainder of the storage period was fairly constant at 5° C. (41° F.), seldom varying from this temperature more than 1° in either direction. The relative humidity in the air-cooled plant ranged from 40 to 90 per cent, the daily variations often covering a large part of this range. The average relative humidity was approximately 65 per cent.

TABLE IX.—*Apple-scald in air-cooled storage: Experiment at Winchester, Va.*

Lot No.	Treatment.	Percentage of scald.			
		Ventilated barrels.	Commercial barrels.		
		York Imperial.	York Imperial.	Arkansas.	Yellow Newtown.
1	Cold storage, in aisle	1	25	50	10
2	Air-cooled storage Oct. 31 to Dec. 17, and cold storage the rest of the storage period	.....	45	60	.....
3	Air-cooled storage Oct. 31 to Nov. 26, and cold storage the rest of the storage period	3	25	55	35
4	Air-cooled storage Nov. 26 to Dec. 17, and cold storage the rest of the storage period	1	40	30	20

The experiments recorded in Table X were made in Wenatchee, Wash. The Grimes apples were picked September 18, and the Yellow Bellflower September 21. The notes on both were taken February 15. The Rome Beauty apples were picked October 2, and the Stayman Winesap October 4. Both were removed from storage March 17 and notes taken March 25. One box of each variety was used under each storage condition. The average temperature of the cold-storage plant was 5° C. (41° F.) during September and October, 1.8° C. (35.2° F.) during November, and 0° C. (32° F.) during the remainder of the storage period. The relative humidity ranged between 80 and 90 per cent, averaging approximately 85 per cent for the entire storage period. In the air-cooled plant the average temperature during September and October was 12.2° C. (54° F.),

during November 3.3° C. (38° F.), during December 2.2° C. (36° F.), and for the remainder of the storage period 0.8° C. (33.4° F.). In the air-cooled cellar the temperatures were those given in paragraph 4, page 220. All the apples were moved to a temperature of 20° C. (68° F.) one week before the final notes were taken.

TABLE X.—*Apple-scald in air-cooled storage: Experiments at Wenatchee, Wash.*

Lot No.	Treatment.	Percentage of scald in boxes.					
		Grimes.	Rome Beauty.	Stayman Winesap.	Yellow Bellflower.	Grimes.	
						Heavily irrigated.	Lightly irrigated.
1	Cold storage	35	11	12	15	69	23
2	Air-cooled storage	30	6	.....	0	59	17
3	Cold storage 1 month, then air-cooled storage	42	.....	4	.....	.....	.....
4	Air-cooled storage 1 month, then cold storage	7	.....	0	.....	.....	.....
5	Cold storage 2 months, then air-cooled storage	40	10	[a]14	.....	.....	.....
6	Air-cooled storage 2 months, then cold storage	60	7	[b]0	.....	.....	
7	Air-cooled cellar storage	.....	.....	.....	.....	83	22

[a] Cold storage 4 months. [b] Air-cooled storage 4 months.

In the West Virginia experiment, the apples in air-cooled storage scalded worse than those in cold storage, while in the experiment at Wenatchee, Wash., the apples in cellar storage were scalded most, the ones in cold storage next, and those in air-cooled storage least. The results appear to be contradictory, but are really in harmony with the fundamental facts. It was pointed out earlier in the paper that scald development is decreased by low temperatures and also by aeration. The temperatures in the cellar storage at Wenatchee and in the air-cooled plant at Gerrardstown, W. Va., were higher than those in the air-cooled plant at Wenatchee; and the air circulation in the first two places was also poorer than that in the last. So while the results reported in the table appear contradictory so far as air-cooled storage is concerned, they are in harmony with the laws of scald occurrence. Air-cooled storage conditions vary greatly with the weather and with the construction and management of the storage house, and the results on scald will necessarily vary accordingly. The infrequency of cool nights in the fall of 1918 made the management of air-cooled houses unusually difficult.

COLD-STORAGE SYSTEMS AND METHODS

Experiments were made at Wenatchee, Wash., and Winchester, Va., to determine the effect of ventilation and aeration in commercial cold-storage plants. The apples were held at 0° C. (32° F.) or slightly above

in all the different tests. In the Wenatchee experiment part of the apples were stored in a room cooled by direct expansion and the others in a room cooled by the bunker system. In the former experiment there was practically no air movement, while in the latter the apples were stored at a distance of about 3 feet from an opening in the outgoing air duct and were constantly fanned by an air current moving at the rate of 0.88 miles per hour. Two boxes of apples of each variety were used under each storage condition. The Grimes were stored September 18, the Stayman Winesap October 12, and the Rome Beauty October 2. All were in the direct expansion storage room till October 21, when half of each lot was moved to the bunker system storage. The final notes on the Grimes were taken February 15 and on the Stayman Winesap and Rome Beauty March 25. All the apples were held at a temperature of 20° C. (68° F.) for four days before the notes were taken.

TABLE XI.—*Apple-scald in direct expansion and bunker systems of cold storage*

Kind of storage.	Percentage of scald.		
	Grimes.	Stayman Winesap.	Rome Beauty.
Direct expansion	35	12	11
Bunker	1	0	8

The results would indicate that the bunker system was much more favorable to scald prevention than the direct expansion. It should be noted, however, that with the bunker storage the apples were given one of the most favorable locations in the room so far as air circulation was concerned. Anemometer readings showed that the air in the lower corners of the room was practically stagnant and but little affected by the air circulation above. What the results in Table XI do show is that a continuous air circulation at the rate of 0.88 mile per hour practically eliminates scald on box apples.

In the experiment at Winchester, Va., all the apples were stored in large rooms cooled by a direct expansion system, but the different lots were variously located so as to receive different amounts of aeration. One of the storage rooms had two outside windows, each 3 feet wide and 5 feet high, in the west wall of the room, and two similar windows in the east wall. The doors in the elevator shaft were near the east end of the room. The windows and doors were thrown open on cool nights and the outside air admitted freely into the storage room. A $\frac{1}{10}$ horsepower ventilating fan was sometimes used in one of the doors. Such breezes as were obtained were so soon dissipated that it was never possible to obtain anemometer readings at a greater distance than 10 feet from any of the windows.

Because of the infrequency of cool nights in the fall of 1918 and the difficulty of having somebody at the storage rooms at the right time, only five ventilations were given during the critical period for scald. The first of these was made on November 12, and the others followed at weekly intervals. Considerable benefit was apparently derived from these ventilations, but probably not as much as from the daily fanning of the doors in connection with the regular storage-house operations. The apples of lot 1, Table XII, were stored in a corner of the room in the bottom of the stack, those of lot 2 near a west window in the middle of a large stack, and those of lot 3 in an aisle between an east window and the door into the elevator shaft. The apples of lot 4 were in an aisle near the door of a second storage room that was similar to the first but had no windows and received no special ventilations. The Stayman Winesap apples were stored September 25, and the final notes taken January 23; the Arkansas stored October 28, and the final notes taken January 30; and the York Imperial stored October 1 and October 29, and the final notes taken February 14 and March 8. Three barrels of each variety were used under each storage condition.

TABLE XII.—*Aeration in commercial cold storage*

Lot No.	Storage location.	Percentage of scald.					
		York Imperial.				Stayman Winesap.	Arkansas.
		Stored Oct. 1.		Stored Oct. 29.			
		Ventilated barrels.	Commercial barrels.	Ventilated barrels.	Commercial barrels.	Commercial barrels.	Commercial barrels.
1	In corner in bottom of stack	.....	80	.....	.....	.....	.....
2	Near window in middle of stack	15	44	.....	.....	49	.....
3	In aisle near window	12	32	1	19	32	28
4	In aisle of unventilated room	15	35	5	28	67	50

Of the first three lots of apples, all from the same room, those in the bottom of the stack at a distance from the windows and doors were scalded practically twice as much as any of the others; those surrounded by other barrels but near a window were next, while those in an aisle near a window had least scald. The apples of lot 4 which were in the aisle of the unventilated room were, in general, much worse scalded than those of lot 3, which were in the aisle of the ventilated room.

The results of the two experiments show a very close relationship between air circulation in cold-storage plants and scald prevention. It seems evident that the general management of the rooms and the arrangement of the stacks and the aisles are important factors in securing aeration of the fruit. The renewal of the air in the storage room is

minor importance compared with sufficient stirring of the air within the room to enable the apples to throw off their waste gases.

STORAGE PACKAGES

In a question where the aeration of the fruit is involved, the nature of the package naturally plays an important part. In an earlier paper (5) preliminary experiments were reported covering a number of different temperatures and a great many different varieties of apples and showing that scald could always be produced by storing the apples in moist chambers and could always be prevented by storing them in open containers. Preliminary experiments were also reported showing that scald could be reduced in commercial storage by the use of ventilated barrels. The results reported in the following paragraph give confirmatory evidence of the importance of open packages in commercial storage.

The data reported in Table XIII were obtained in Wenatchee, Wash. The Grimes apples were picked September 18, were held in a laboratory until October 4, and were then placed in cellar storage. The lard cans remained closed until the apples were removed from storage. The final notes were taken February 14. The Rome Beauty apples were picked October 3, were held in cellar storage till November 2, and were then placed in cold storage. The lard cans remained closed till the apples were removed from storage. The final notes were taken February 14. The Stayman Winesap apples were picked October 12, were held in a cool, well-ventilated place till October 19, and then the lard cans were opened and all of the apples transferred to cold storage. The final notes were taken March 25. All lots were held in the open in a warm laboratory for three or more days before the final notes were taken. The following results were obtained on unwrapped apples, but similar contrasts were also obtained on wrapped apples.

TABLE XIII.—*Closed packages in storage*

Variety.	Percentage of scald.	
	In lard cans.	In commercial boxes.
Grimes	42	24
Rome Beauty	68	25
Stayman Winesap	34	2

The results show that the tight package greatly increased the amount of scald.

The experiments reported in Table XIV were made in Winchester, Va., and Washington, D. C. The Grimes at Winchester were picked September 11, the Stayman Winesap September 25, the green York Imperial October 1, and the ripe York Imperial October 29. The Grimes at

Washington, D. C., were picked September 3, the Rome Beauty September 27, and the York Imperial October 10. The Grimes and Rome Beauty in the Washington experiment were from Franklin, Va., and the York Imperial from Greenwood, Va. The time of taking the final notes is given in the table. Three baskets and from 3 to 15 barrels of apples were used under each storage condition reported. The baskets held approximately a bushel of apples each and were of the low, tight form with overlapping slats. The ventilated barrels were made by cutting holes in the staves of the usual commercial barrels. Fifteen holes ¾ inch by 4 inches were made in each barrel, care being taken to have the openings well distributed and to avoid weakening the barrel by making cuts too near the bulge. A more satisfactory barrel can be obtained by having the cooper notch the staves before the barrel is made. The room in which the Winchester apples were stored received an occasional ventilation, while that in which the Washington apples were stored did not; but in both cases the apples were near the door.

TABLE XIV.—*Influence of baskets and ventilated barrels upon apple-scald*

Treatment.	Percentage of scald.						
	Ventilated room, Winchester, Va.				Unventilated room, Washington, D. C.		
	Grimes, Jan. 11.	Stayman Winesap, Jan. 23.	York Imperial.		Grimes, Dec. 20.	York Imperial, Jan. 23.	Rome Beauty, Jan. 28.
			Green, Feb. 14.	Mature, Feb. 20.			
Immediate storage:							
Commercial barrels	33	40	45	25	35	20	28
Ventilated barrels	8	16	15	1	30	8	20
Baskets	.....	18	15	.....	.....	.....	.....
Delayed storage:							
Commercial barrels	29	76	65	.....	12	60	80
Ventilated barrels	20	.....	35	.....	3	17	0
Baskets	.....	25	45	.....	.....	.....	.....

The results with the baskets were similar to those with the ventilated barrels. With the immediate storage in the ventilated room, the ventilated barrels in every case reduced the scald to at least one-third of that in the commercial barrels; but with the fruit in the unventilated room, the ventilated barrels caused only a slight reduction in scald. In the delayed storage, the ventilated barrels resulted in a great decrease in scald in nearly every case, often reducing the percentage of the disease below that in the immediately stored commercial barrel. The results, as a whole, show that the ventilated barrel can be used to great economic advantage in the prevention of apple-scald.

The better aeration of the ventilated barrels was evidenced in the quicker rate of cooling on going into storage and in the composition of

the air of the barrels as well as in the prevention of apple-scald. These facts are brought out in a graphic manner in figures 1 and 2.

In the tests on rate of cooling, the temperature records were obtained by means of resistance thermometers, the thermometer bulbs being forced into apples in the middle of the barrels and the readings taken from the outside with an indicator without disturbing the fruit.

It will be noted from the curves in figure 1 that during the first few days in storage the apples in the ventilated barrels were from 5° to 10° F.

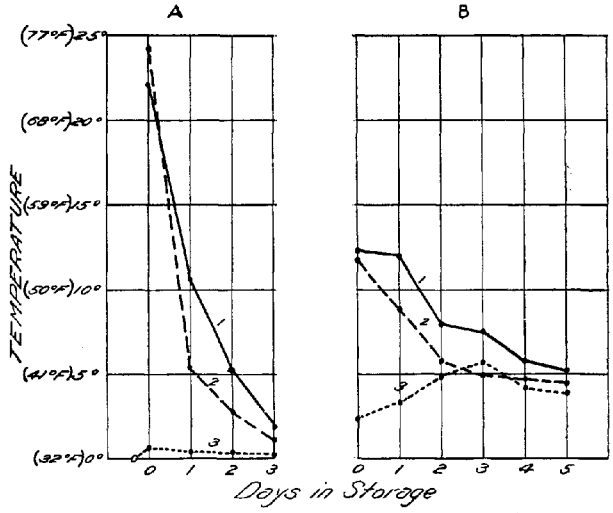

FIG. 1.—Relative rate of cooling of apples in commercial and ventilated barrels: A, Grimes apples in a storage room already filled with cold fruit; B, York Imperial apples in a storage room still receiving a large bulk of warm fruit. Curve 1 shows the temperature of the fruit in the center of a commercial barrel; curve 2, the temperature of the fruit in the center of a ventilated barrel; and curve 3, the temperature of the storage room.

colder than those in the commercial barrels. The quicker cooling secured by the ventilated barrels has a value in itself; but since the cooling is accomplished by air currents, the temperature contrast is also of interest as proof of the much freer exchange of air allowed by the more open barrels.

The relative carbon-dioxid content of the air in the ventilated and commercial barrels during the first weeks of storage is shown in figure 2. The gas analyses in figure 2, A, were made with the Pettersson gas apparatus and those in figure 2, B, with the Allen-Moyer Orsat apparatus. The samples were taken from the center of the barrel, small tubes having been arranged for this purpose at the time the apples were packed. A study

of the curves shows that there was usually more than twice as much carbon dioxid in the air of the commercial barrels as in the air of the ventilated barrels and but little more in the air of the ventilated barrels than in the air of the storage room. As was pointed out earlier in the paper (p. 213) small quantities of carbon dioxid do not appear to be harmful to apples; but since the fruit is continually giving off this gas, the quantity of it in the storage air does serve as an indicator of the extent of ventilation. The results give further evidence of a decided contrast between the aeration secured in the ventilated and commercial barrels.

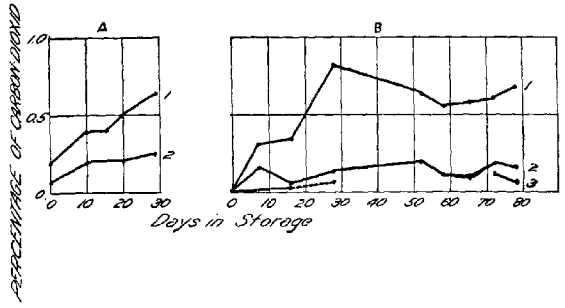

FIG. 2.—Relative carbon-dioxid content of the air in ventilated and commercial barrels during the first weeks of storage: A, Grimes apples in a storage room already filled with cooled fruit; B, York Imperial apples in a storage room still receiving a large bulk of fresh fruit. Curve 1 shows the percentage of carbon dioxid in the air of a commercial barrel; curve 2, the percentage in the air of a ventilated barrel; and curve 3, the percentage in the air of the storage room.

WAXES, FATS, OILS, AND OTHER GAS ABSORBENTS AS AGENCIES IN SCALD PREVENTION

Preliminary experiments were reported in an earlier paper (5) indicating that certain waxes and oils could be used as absorbents for the gases that are instrumental in producing apple-scald. In the following experiments the earlier results are confirmed and the list of gas absorbents greatly extended.

The neutral mineral oil wrappers were obtained from an oiled manila paper similar to that used in meat markets. The paraffin wrappers A were made by saturating ordinary apple wrappers with paraffin; the paraffin wrappers B and D were made from very light-weight commercial paraffin paper; and the paraffin wrappers C from a fairly heavy commercial paraffin paper. The glassine wrappers were from paper sold commercially under that name and apparently contained no wax or oil. All the other wrappers reported in Tables XV, XVI, and XVII were prepared by saturating the usual commercial apple wrappers with the given oil or wax.

The results reported in Table XV were obtained in experimental storage boxes, the separate lots of apples being held in moist chambers. Only 10 apples were used in each lot, but great care was taken that the different lots should be as nearly alike as possible. The tests were carried out under several different conditions with remarkably consistent results. The Grimes of August 21 were quite green and were placed under the given storage conditions the day after picking. The Grimes of October 29 were picked September 4, packed in barrels, and held in commercial cold storage from September 5 to October 28. The Rhode Island Greening apples were held in barrels in cold storage till November 5, and the Yellow Newtown apples till December 19. The wrapper experiments on the last three lots were started at the time the apples were removed from cold storage.

TABLE XV.—*Effect of oils, waxes, and other gas absorbents upon apple-scald*

Wrappers or packing.	Percentage of scald.								
	Grimes, Aug. 21, 1918.				Grimes, Oct. 29, 1918, after 14 weeks at—			Rhode Island Greening, after 10 weeks at 15° C.	Yellow Newtown after 14 weeks at 5° C.
	After 10 weeks at—		After 16 weeks at—						
	15° C.	10° C.	2½° C.	0° C.	5° C.	2½° C.	0° C.		
None, control	60	40	43	58	90	52	53	85	40
Wrappers, commercial	45	.....	38	50	63	55	70	42	35
Wrappers, paraffin A	12	.....	.....	20	60	25	35	.....	6
Wrappers, paraffin B	12	.....	15	30	.....	.....	.....	.....	.....
Wrappers, paraffin C	15	.....	32	38	.....	.....	.....	.....	.....
Wrappers, glassine	.....	.....	.....	.....	.....	.....	.....	70	50
Wrappers beeswax 30 per cent, vaseline 70 per cent	0	.....	3	30	3	10	3	.....	.....
Wrappers, beeswax 30 per cent, olive oil 70 per cent	1	.....	32	23	.....	.....	.....	.....	0
Wrappers, cacao butter 70 per cent, vaseline 30 per cent	1	.....	20	18	.....	.....	.....	.....	.....
Wrappers, cacao butter 70 per cent, olive oil 30 per cent	.....	.....	12	18	.....	.....	.....	.....	.....
Wrappers, olive oil	.....	.....	.....	.....	.....	.....	.....	.....	0
Wrappers, neutral mineral oil	0	.....	5	9	.....	.....	.....	.....	0
Wrappers, vaseline	0	.....	18	18	.....	.....	.....	0	0
Wrappers, cacao butter	3	.....	20	35	.....	.....	.....	0	.....
Wrappers, beeswax	.....	.....	.....	.....	.....	.....	.....	9	0
Wrappers, ceresin wax	.....	.....	.....	.....	.....	.....	.....	.....	0
Wrappers, Japanese wax	.....	.....	.....	.....	.....	.....	.....	.....	0
Wrappers, Carnauba wax	.....	.....	.....	.....	.....	.....	.....	.....	10
Wrappers, apple wax	.....	.....	.....	.....	.....	.....	.....	.....	0
Wrappers, ivory soap	.....	.....	.....	.....	.....	.....	.....	.....	10
Wrappers, glycerin	.....	.....	.....	.....	.....	.....	.....	.....	30
Wrappers, sugar, 100 per cent solution	.....	.....	.....	.....	.....	.....	.....	.....	30
Wrappers, lard	.....	.....	.....	.....	.....	.....	5	17	0
Wrappers, tallow	.....	.....	.....	.....	.....	.....	7	0	0
Wrappers, cow butter (unsalted)	.....	.....	.....	.....	.....	.....	4	0	0
Wrappers, linseed oil	.....	.....	.....	.....	.....	.....	.....	30	.....
Wrappers, neat's-foot oil	.....	.....	.....	.....	.....	.....	.....	0	.....
Wrappers, cottonseed oil	.....	.....	.....	.....	.....	.....	.....	40	.....
Wrappers, corn oil	.....	.....	.....	.....	.....	.....	.....	20	.....
Wrappers, peanut oil	.....	.....	.....	.....	.....	.....	.....	.....	0
Wrappers, castor oil	.....	.....	.....	.....	.....	.....	.....	.....	0
Charcoal, animal	15	35	.....	.....	.....	.....	.....	.....	.....
Charcoal, wood	30	35	.....	.....	.....	.....	.....	.....	.....
Charcoal, lignite	60	30	.....	.....	.....	.....	.....	.....	.....
Cork, granulated, wet	7	40	.....	.....	.....	.....	.....	.....	.....
Cork, granulated, dry	1	.....	.....	.....	.....	.....	.....	.....	.....
Excelsior	30	.....	.....	.....	.....	.....	.....	.....	.....
Brick, granulated	30	.....	.....	.....	.....	.....	.....	.....	.....
Pumice stone, granulated	45	15	.....	.....	.....	.....	.....	.....	.....
Oatmeal	5	20	.....	.....	.....	.....	.....	.....	.....

Linseed oil and castor oil injured the skin of the apples wherever the wrapper was in close contact with the fruit.

The tests reported in Table XVI were made in a commercial cold-storage plant at Wenatchee, Wash. The apples were packed in boxes, one layer of apples being used for each treatment with a thick layer of newspapers between lots. The Grimes apples were stored September 18, were removed to a temperature of 20° C. (68° F.) on February 6, and the final notes taken February 15. The Stayman Winesap apples were stored October 12 and the Rome Beauty October 10. The Stayman Winesap apples were in storage two weeks before the fruit was wrapped. The apples of both these varieties were removed to a temperature of 20° C. (68° F.) on March 15 and the final notes taken March 24.

TABLE XVI.—*Box apples wrapped and unwrapped*

Kind of wrapper.	Percentage of scald.		
	Grimes.	Rome Beauty.	Stayman Winesap.
None, control	75	12	16
Commercial, no wax or oil	60	.....	19
Paraffin B	.....	.....	11
Paraffin C	.....	.....	17
Paraffin D	.....	6	.....
Glassine	.....	.....	47
Paraffin 50 per cent, vaselin 50 per cent	0	1	.....
Vaseline	2	0	.....
Beeswax 30 per cent, vaseline 70 per cent	0	0	.....
Cacao butter 70 per cent, vaseline 30 per cent	5	0	.....
Beeswax 30 per cent, olive oil 70 per cent	0	3	.....
Cacao butter 75 per cent, olive oil 25 per cent	0	1	.....
Cacao butter	0	.....	.....
Mineral oil	.....	.....	0

The tests reported in Table XVII were made with eastern barreled apples. Only about one-third of the apples of the barrel were wrapped. The barrels were filled approximately half full of unwrapped apples, a bushel of wrapped apples added, and the remainder of the barrel filled with unwrapped apples. The Grimes were from Vienna, Va., and were placed in commercial cold storage at Washington, D. C., on September 18. The York Imperial were from Winchester, Va., and were placed in commercial cold storage at that point on October 1. The apple-scald notes on the Grimes were taken December 20 and those on the York Imperial March 12. Both lots were held at a temperature of 20° C. (68° F.) for a period of three days before the notes were taken. See Table XVII.

The results in Tables XV, XVI, and XVII give conclusive proof that there is a wide range of materials that are capable of absorbing the harmful substances produced in apple storage. With some of the materials, such as oatmeal and granulated cork, a part of the good

effects might possibly be attributed to the influence upon humidity; but the results as a whole require a different explanation. As was pointed out earlier in the paper (p. 216) it seems practically certain that the beneficial effects, particularly of the waxes, fats, and oils, are due to their power of absorbing esters or other similar products thrown off in gaseous form by the apple.

TABLE XVII.—*Apple wrappers in commercial barrel storage*

Kind of wrapper.	Percentage of scald.					
	Grimes.			York Imperial.		
	Wrapped.	Not wrapped.	Next layer to wrapped apples.	Wrapped.	Not wrapped.	Next layer to wrapped apples.
None, control barrels		38			40	
Commercial, no wax or oil	27		30	28		30
Paraffin A	18		33	35		30
Paraffin B	15		23	10		30
Paraffin C	23		32	20		
Beeswax 30 per cent, vaselin 70 per cent	9		23	2		35
Beeswax 30 per cent, olive oil 70 per cent	18		33	5		18
Cacoa butter 70 per cent, olive oil 30 per cent	8		32	1		12
Mineral oil	0		18			

The results furnish some very interesting contrasts. In most tests the commercial wrappers caused little or no reduction in scald, and the paraffin wrappers were but little better, while nearly all the other wrappers caused a decided decrease in the disease. Particularly good results were obtained with fats like cow butter and tallow and with neat's foot oil and mineral oils. It should be noted that there is a close correlation between the ability of the various substances to control apple-scald and their capacity for absorbing gases.

A very significant point is brought out in Table XVII in the extension of the scald reduction to the apples that were adjacent to the wrapped ones. With some of the more efficient wrappers the scald on the contiguous fruit was reduced to less than one-half of that on the fruit in other barrels or in the distant parts of the same barrel. In most cases this effect extended only to apples actually in contact with the wrapped apples, but with the olive and mineral oil wrappers there was an evident decrease in scald at a distance of several layers from the wrapped fruit. These results can hardly be explained by any other theory than that the good effects of the wrappers are largely due to the gas-absorbing capacity of the fats and oils they contain.

PRACTICAL CONSIDERATION

In recording the percentages of scald in the foregoing experiments, consideration has been given to both the number of apples affected and the intensity of the disease; the scald ratings therefore bear a very close relation to the actual damage done to the fruit and to the reduction in price resulting from it. In an average market, the loss in price on the apples would be about half that of the percentage of scald recorded—for example, apples that have been marked as having 80 per cent of scald would ordinarly be sold at a reduction of about 40 per cent in price, apples having 50 per cent of scald at a reduction of 25 per cent, and apples having 5 or 10 per cent of scald at little or no reduction. It is possible, therefore, to obtain a fairly close estimate of the effect of the various treatments upon the value of the fruit. It will be seen by reference to Table XII that barreled apples stored in the bottom of the stack at a distance from the window were damaged by scald to the extent of 40 per cent of their value (80 per cent of scald), while similar apples near the window or in the aisles were damaged but 15 per cent, and the apples in the ventilated barrels but 6 per cent—an amount that might be entirely overlooked in many markets. In the wrapper experiments as shown in Tables XV, XVI, and XVII, the unwrapped apples and those in commercial wrappers were damaged by scald to the extent of from 20 to 40 per cent of their value while those wrapped in the best of the waxed papers were practically free from injury.

These estimates of damage are based on the assumption that the fruit becomes warm before being used. If the apples were sold on a northern market in cold weather, the loss from scald might not be felt by the dealer but be largely passed along to the consumer; but if it were necessary to expose the fruit in moderately warm weather the loss would be shown in the actual selling price. Whether the scald damage becomes evident on the market or only after the fruit has passed to the hands of the consumer, the loss is a real one. Apples that should have remained in good condition for several weeks under common storage conditions are rendered unfit for anything but immediate consumption and even undesirable for that. Not only does the scalded condition gradually spread to considerable depth in the tissue of the apple but the death of the skin exposes the softer tissues beneath to the action of blue mold (*Penicillium expansum*) and other rot organisms, and rapid decay follows. Apples with a sound epidermis are practically immune to rot at high as well as low temperatures (*3*), but apples with the skin killed by scald are doomed to early destruction.

In the apple trade the time at which scald appears on the fruit receives more consideration than the severity of the disease when it occurs. Anything that will postpone the development of apple-scald means a

greater freedom in marketing and fewer rush sales. In the experiments that have been reported no statements have been made as to the time when scald first appeared on the different lots of fruit, but a record was kept of this whenever possible. In the experiment reported in Table XII the York Imperial apples in commercial barrels in the aisle had 32 per cent of scald on January 28, and those in ventilated barrels 10 per cent of scald. On March 12, approximately 6 weeks later, the apples in the ventilated barrels had increased in scald, but only to 18 per cent, and, as is shown in Table XVII, the apples of this same lot that were in the best grade of wrappers were still entirely free from scald. It was impossible to obtain an early record of the apples in commercial barrels in the bottom of the stack (Table XII); but judging from the usual rate of development and the fact that they had 80 per cent of scald on January 28 it is probable that they had 20 to 30 per cent of scald by January 1. In other words, York Imperial apples in commercial barrels in the bottom of the stack were scalded badly enough to have their market value affected by January 1; similar apples in the aisle did not reach the same degree of scald till 4 weeks later; those in ventilated barrels in the aisle had scarcely reached it at the end of 10 weeks; and apples in waxed wrappers were entirely free from scald at the end of this period. What this means to the trade can be readily seen. On the one hand, apples must either be sold so early as to be out of season, or else disposed of for immediate consumption at a later date; on the other hand, if the fruit receives sufficient aeration in storage or is protected by oiled wrappers the dealer may choose his own time for selling and can expose his fruit on the market or ship it to distant points without fear of its going down with scald.

A study of the market products as they pass to the consumer will convince anyone of the enormous food and money losses resulting from apple-scald. It is the opinion of the writers that with the present method of handling apples the losses from this disease are greater than those from all other transportation and storage diseases of the apple, but in spite of all this direct loss it seems to them that the greatest injury to the apple trade comes from the effects of scald upon public confidence. A dealer or consumer buys with the assurance that the apples are of high quality and in good condition, and the seller may really believe them to be; but if the fruit becomes somewhat warmer before the buyer has an opportunity to inspect it he finds a scalded, rotten-looking lot of apples and naturally concludes that he has been cheated. At the present time there is much discussion as to the best methods of increasing apple consumption by increasing exports to foreign countries, extending the trade in the South, and increasing the shipments to small cities that can not handle car load lots. Apple-scald is one of the great barriers to this trade expansion, the disease not only often making it impossible to deliver

fruit in good condition but serving also as a continual source of misunderstanding.

The actaul losses caused by scald and the uncertainty it introduces into the apple trade add greatly to the cost of market operation and help to widen the gap between the producer's and the consumer's prices. The foregoing experiments show that it is a preventable disease and that with proper methods of handling the apples in the orchard and in transportation and storage the disease can be reduced to a negligible quantity if not entirely eliminated.

SUMMARY

(1) The foregoing experiments show that the occurrence of apple-scald is determined by orchard, packing house, transportation, and storage conditions.

(2) As has been shown by other investigators, mature fruit has in general scalded less than immature; but it has also been found that the fruit surfaces just changing from green to yellow have scalded worse than those that were a leaf green and worse than those that had more completely changed to yellow. Well-colored red fruit surfaces have been practically immune to scald.

(3) Apples from trees receiving heavy irrigation have scalded worse than those from trees receiving light irrigation. This was found not to be due to the greater number of large apples in the former case but to some forcing effect that increased the susceptibility to scald in both large and small apples.

(4) Delayed storage has increased or decreased apple-scald, depending upon the amount of aeration the apples received during delay.

(5) Apples in ventilated barrels have developed less than one-third as much scald as those in commercial barrels when both were held in a storage room that received an occasional ventilation, but where the storage room received little or no ventilation the ventilated barrels caused but little decrease in scald.

(6) The amount of scald developed in cold-storage plants has varied greatly with the location in the room. Apples near the aisle or near a door have scalded far less than those in the bottom of the stack. Boxed apples exposed to a continuous air current of 0.88 mile per hour in a commercial storage plant have been practically free from scald, while similar apples that did not receive the constant fanning became badly scalded. Stirring of the storage air has been found more important than its renewal in the prevention of apple-scald.

(7) The ordinary commercial apple wrappers have caused but little decrease in scald, and paraffin wrappers have been but slightly better, but wrappers impregnated with various fats and oils have either entirely prevented the disease or reduced it to a negligible quantity. In barrels

experiments in which only part of the fruit was wrapped, scald has been greatly reduced on the apples adjacent to the wrapped ones as well as on the wrapped apples themselves.

(8) Typical scald has been artificially produced in a few days' time by exposing apples to the vapors of ethyl acelate, amyl acetate, or methyl butyrate.

(9) The manner in which scald can be produced artificially and the different methods of control indicate that the disease is due to the accumulation of esters or similar products of the apple in the tissues of the fruit and in the surrounding air. The vapors of these substances can be carried away by air currents or absorbed by fats and oils.

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